The fate and effects of lead (Pb) at active and abandoned shooting ranges in a boreal forest ecosystem

Salla Selonen

Department of Environmental Sciences
Faculty of Biological and Environmental Sciences
University of Helsinki
Lahti
Finland

Academic dissertation in Environmental Ecology

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in the Auditorium of Lahti Science Park, Niemenkatu 73, Lahti, on April 10th, at 12 noon.

Lahti 2015

Supervisors Professor Heikki Setälä
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

Professor Rauni Strömmer
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

Reviewers Doctor Jack H. Faber
Alterra, Wageningen University and Research Centre
Wageningen, The Netherlands

Professor Cornelis A. M. van Gestel
Department of Ecological Science
VU University Amsterdam
Amsterdam, The Netherlands

Opponent Professor Juliane Filser
General and Theoretical Ecology
Center for Environmental Research and
Sustainable Technology (UFT)
University of Bremen
Bremen, Germany

Custos Professor Heikki Setälä
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

ISBN 978-951-51-0906-4 (paperback)
ISBN 978-951-51-0907-1 (PDF; http://ethesis.helsinki.fi)
ISSN 1799-0580

Unigrafia
Helsinki 2015

Omistettu
Matiakselle, Viljamille ja Inkerille

CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to by their Roman numerals in the text:

I. Selonen, S., Liiri, M., Strömmer, R. & Setälä, H. 2012: The fate of lead at abandoned and active shooting ranges in a boreal pine forest. Environmental Toxicology and Chemistry 31: 2771-2779.

II. Selonen, S., Liiri, M. & Setälä, H. 2014: Can the soil fauna of boreal forests recover from lead-derived stress in a shooting range area? Ecotoxicology 23:437-448.

III. Selonen, S. & Setälä, H. Long-term changes in nutrients and microbes in lead contaminated boreal forest soil at shooting ranges. Submitted manuscript.

IV. Selonen, S. & Setälä, H. 2015: Soil processes and tree growth at shooting ranges in a boreal forest reflect contamination history and lead-induced changes in soil food webs. Science of the Total Environment 518-519:320-327.

THE AUTHOR'S CONTRIBUTION

Salla Selonen (SS) planned the field surveys together with the other authors and conducted the field samplings. SS is the corresponding author of all articles in this thesis and wrote the articles with contributions from the other authors. SS also contributed to the individual studies as follows:

I. SS analysed Pb and other soil properties from the soil samples. She instructed Pb extractions from plants, participated in analysing Pb in plants and microarthropods, and performed the statistical analyses.

II. SS performed the faunal extractions and soil analyses, and counted the enchytraeid worms and total number of nematodes. She counted and identified microarthropods with the assistance of Mira Liiri, and performed the statistical analyses.

III. SS performed nutrient extractions from the soil samples. She also conducted the pre-treatments of leachate samples for nutrient analyses and of soil samples for PLFA analyses. SS performed the statistical analyses and analysed the PLFA data with the assistance of Mira Liiri and Gaia Franzini.

IV. SS conducted the litter bag experiment and measured monthly litter production and tree radial growth. SS participated in the faunal, microbial and soil analyses as described above and performed the statistical analyses.

ABSTRACT

Despite the known toxicity of lead (Pb) and the ban on waterfowl hunting, Pb pellets are still used at shotgun shooting ranges around the world. After firing a shotgun, pellets spread across wide areas, ending up in nearby ecosystems, which typically are forests in Finland. Still, little is known about the effects of Pb in these ecosystems and hardly anything about changes in ecosystem structure and function after range abandonment. Thus, ecosystem-level research was conducted in a shotgun shooting range area to evaluate the fate and effects of Pb in a boreal forest ecosystem and the possible recovery of the system after range abandonment.

Bioaccumulation, the leaching and vertical distribution of Pb in the soil, soil nutrients and their leaching as well as structure and activity of decomposer community were studied at two contaminated sites (active [NC] and abandoned [OC] shooting ranges) and a control site, each locating in the same pine forest stand. Furthermore, tree growth, nutritional status and litter production were measured. Total Pb pellet burdens at the contaminated sites were similar, reaching up to 4 kg m^{-2}, and shooting activity had lasted for 20 years at both sites, but occurred 20 years earlier at OC.

Total Pb concentrations at the shooting ranges were extremely high, and Pb accumulated in the biota. The vertical distribution of Pb in the organic soil horizon differed between the shooting range sites, with total Pb concentrations at NC being higher in the upper F layer than in the lower H layer, but vice versa at OC. Soil fungi and all studied faunal groups (enchytraeid worms, microarthropods and nematodes) except protozoans were affected negatively by Pb. Lead decreased phosphate (PO_4^{3-}) and increased nitrate (NO_3^-) concentrations. pH was also increased by Pb, which can further affect the biota directly or indirectly by changing Pb availability and toxicity. In the entire organic soil horizon, the negative effects of Pb were less pronounced at OC than at NC. In addition, pine needle litter decomposed faster at OC than at NC, and tree (*Pinus sylvestris*) radial growth was suppressed at NC after shooting activity started and increased at OC after shooting activity ceased. However, in the H layer the effects were stronger at OC, enchytraeid worms being completely absent. Furthermore, leaching of Pb through the organic soil horizon was twice as high at OC as at NC.

A decrease in total Pb concentrations and toxicity in the topmost soil layer and enhanced litter decomposition rate at the abandoned shooting range indicate an on-going recovery process. In boreal forest soils that are characterized by low decomposition rates and little soil mixing due to the scarcity of earthworms, a less contaminated soil layer is gradually formed when shooting activities cease. This topmost soil layer can provide habitat for the decomposer biota and promote the recovery of soil functions. However, at the same time the dissolution of Pb from pellets deeper in the soil increases toxicity of the humus and the leaching of Pb, increasing risks to the ecosystem and groundwater quality. These findings suggest that communities and functions in Pb-contaminated boreal forest ecosystems depend on contamination history. However, despite Pb-induced changes in the decomposer communities, only slight changes in ecosystem processes were detected. This indicates high resistance of boreal forest ecosystems to this type of stress.

TIIVISTELMÄ

Lyijyn on todettu olevan eliöille haitallista, ja vaikka lyijyhaulien käyttö vesilintujen metsästyksessä on kielletty, niitä käytetään edelleen ampumarata-alueilla maailmanlaajuisesti. Ampumaradoilta haulit pääsevät helposti leviämään ympäröivään luontoon, joka Suomessa yleensä on kangasmetsää. Lyijyn vaikutuksista näissä ympäristöissä tiedetään kuitenkin vain vähän, ja vielä vähemmän on tietoa siitä, miten lyijyn vaikutukset muuttuvat ajan myötä ampumistoiminnan päätyttyä, kun lyijyhaulit ovat jääneet maaperään.

Tässä väitöskirjatyössä tutkittiin lyijyn kulkeutumista ja vaikutuksia ampumarata-aluetta ympäröivässä metsäekosysteemissä aina maaperän eloperäisestä maakerroksesta (5 – 8 cm) ja maaperäeliöistä metsän puustoon. Lisäksi tarkasteltiin, miten nämä vaikutukset muuttuvat ajan myötä vertailemalla kahta samassa kangasmetsässä sijaitsevaa tutkimusaluetta. Toiselle näistä alueista kulkeutuu hauleja edelleen käytössä olevalta ampumaradalta (uusi rata), kun taas toisella alueella ampumistoiminta loppui noin kaksikymmentä vuotta ennen tämän tutkimuksen alkua (vanha rata). Molemmilla alueilla oli enimmillään jopa 4 kg lyijyhauleja yhtä neliömetriä kohti.

Eloperäisen maakerroksen lyijypitoisuudet sekä vanhalla että uudella radalla olivat huomattavan korkeita ja kohonneita lyijypitoisuuksia todettiin myös maaperäeläimissä ja alueiden kasveissa. Lyijy oli myös vähentänyt maaperän sienibiomassaa sekä useiden maaperäeläinryhmien runsautta. Vaikka maaperäeliöstöllä on tärkeä merkitys hajotustoiminnassa ja ravinteiden vapauttamisessa edelleen kasvien saataville, lyijyn vaikutukset maaperän hajotustoimintaan olivat vähäisiä. Tämä osoittaa, että systeemi pystyy toimimaan suhteellisen hyvin häiriöstä huolimatta.

Lyijy vaikutti myös eloperäisen maakerroksen ominaisuuksiin: maaperän pH ja nitraattipitoisuus (NO_3^-) kohosivat ja fosfaattipitoisuus (PO_4^{3-}) laski lyijyn vaikutuksesta. Maaperän pH:n nousu voi edelleen vaikuttaa maaperän eliöstöön ja osittain selittää sienibiomassan vähentymistä ja nitraatin lisääntymistä maaperässä. Toisaalta kohonnut pH voi vähentää lyijyn saatavuutta joillekin eliöille.

Uuden ja vanhan radan vertailu osoitti, että lyijy painuu maaperässä ajan myötä syvemmälle. Lyijyhaulit vajoavat vuosikymmenten saatossa ja hautautuvat hajoavasta karikkeesta muodostuvaan eloperäiseen maa-ainekseen. Vähemmän lyijyä sisältävässä pintamaassa maaperän eliöiden runsauden ja karikkeen hajotusnopeuden todettiin lisääntyneen. Toisaalta syvemmällä eloperäisessä maakerroksessa (humuskerroksessa) lyijypitoisuudet olivat ajan myötä kasvaneet lyijyhaulien rapautuessa. Tämän todettiin myös lisänneen haittavaikutuksia maaperäeliöstölle – kangasmetsämaan tärkeimpänä hajottajaeläimenä pidettyä änkyrimatoihin kuuluvaa kunttamatoa ei havaittu vanhan radan humuskerroksessa lainkaan. Lisäksi vanhalla radalla eloperäisestä maa-aineksesta huuhtoutui lyijyä noin kaksi kertaa runsaammin kuin uudella radalla. Tämän väitöskirjatyön tulokset osoittavat, että vaikka pintamaan eliöstö ja hajotustoiminta voivat palautua ajan myötä ampumaradan toiminnan päätyttyä, syvemmällä maaperässä eliöstön tila voi heikentyä ja lyijyn pohjaveteen huuhtoutumisen riski kasvaa.

ABBREVIATIONS

AAS	Atomic absorption spectrometer
ANOVA	Analysis of variance
$CaCl_2$-Pb	Lead fraction extracted with 0.01 M $CaCl_2$
dm	Dry mass
F layer	Fermentation layer (beneath the litter layer and above the humus layer)
GFAAS	Graphite furnace atomic absorption spectrometer
H layer	Humus layer (deepest layer of the organic soil horizon)
H_2O-Pb	Lead fraction extracted with deionized water (H_2O)
ICP-MS	Inductively coupled plasma mass spectrometer
LOQ	Limit of quantification
NMDS	Non-metric multidimensional scaling
NC	New contaminated site, the active shooting range
OC	Old contaminated site, the abandoned shooting range
Pb	Lead
PLFA	Phospholipid fatty acid
RDA	Redundancy analysis
SIR	Substrate induced respiration
SOM	Soil organic matter
tot-Pb	Total Pb, extracted with HNO_3

1. INTRODUCTION

1.1 Community and ecosystem ecotoxicology – at the crossroads of ecotoxicology and ecology

One of the major effects of humans is the introduction of toxic chemicals or metals (i.e. toxicants) to the environment (Newman 2010). Along with other types of anthropogenic disturbances, toxicants can have vast impacts on communities and entire ecosystems. In ecotoxicology, the ultimate research target is to provide knowledge to protect the structure and functioning of ecosystems from the negative effects of toxicants (van Gestel 2012). Yet, the effects of toxicants are commonly studied at the level of individual organisms, even though this information is not directly scalable to the community or ecosystem levels because of numerous interactions between species and their abiotic environment (Clements & Newman 2002, Newman 2010). On the other hand, in the field of ecology, disturbance is mostly considered as a structural change of the habitat, whilst the role of contaminants as a cause of disturbance in the ecosystems is far less studied (Filser 2008).

During past decades, the need for merging ecology into ecotoxicology, or getting more "eco" in ecotoxicology, has become apparent (Van Straalen 2003, Filser et al. 2008, Clements & Rohr 2009). For example, the implementation of functional ecology and ecological resistance and resilience into ecotoxicology has gained increasing attention (Breure et al. 2008, Lahr et al. 2008, Clements & Rohr 2009, Kools et al. 2009, van Gestel 2012, Faber et al. 2013). This thesis takes another step towards unifying ecology and ecotoxicology, by exploring traditional ecological concepts – ecosystem resistance and resilience – from an ecotoxicological perspective.

1.2 Ecotoxicology meets ecology in soils and terrestrial ecosystems

In terrestrial ecosystems, the greatest diversity of the biota is hidden below the ground and is believed to represent one of the most species rich communities that exist (Giller 1996, Coleman et al. 2004, Begon et al. 2005, Binkley & Fisher 2013). This diversity is organised into various trophic and taxonomic groups, forming complex food webs. Organisms within soil food webs interact with each other not only by feeding on or competing for resources, but also by changing the environment and resources due to their activities (Coleman et al. 2004, Begon et al. 2005). Because of these interactions, toxicants can affect various species in the food web also indirectly, by, for instance, changing food resources, competition, predation pressure or the abiotic environment (Clements & Newman 2002, Clements & Rohr 2009).

Linkages between different biota also extend aboveground via various interactions and feedback loops between the two systems (Setälä & Huhta 1991, Wardle et al. 2004, Bardgett & Wardle 2010). The best-known example of such linkages exists between decomposers and primary producers. Since the soil food web plays a fundamental role in decomposition processes and nutrient

cycling, changes in soil communities due to disturbances can be reflected in plant growth and other ecosystem functions aboveground (Heneghan & Bolger 1998, Laakso & Setälä 1999, Filser 2002, Coleman et al. 2004, Bardgett & Wardle 2010).

On the other hand, changes in soil communities due to toxicants or other disturbances are not necessarily reflected in soil processes due to high functional redundancy among the soil biota (Walker 1992, Ehrlich & Walker 1998, Laakso & Setälä 1999, Setälä & McLean 2004, Rohr et al. 2006, Bardgett & Wardle 2010). In other words, a given function can remain if disturbance-tolerant species replace more sensitive ones that carry out the same ecological function. However, not all species are functionally redundant. The loss of species that are functionally important and abundant under normal conditions can have a substantial impact on system functions (Walker 1992, Laakso & Setälä 1999). A key species in boreal forest soils that are studied in this thesis is *Cognettia sphagnetorum*. This omnivorous enchytraeid worm is known to play a crucial role in decomposition processes in acidic, humic-rich soils with low numbers of earthworms (Huhta et al. 1998, Laakso & Setälä 1999).

Changes in community structure, together with acclimation of individuals and genetic adaptation in populations due to the long-term exposure to contaminants may lead to pollution-induced community tolerance (PICT) (Bååth et al. 2005, Puglisi et al. 2012, Berard et al. 2014). However, acclimation or adaptation to a toxicant may have fitness costs and the toxicant may reduce genetic diversity in populations, which can lead to increased susceptibility to other stressors or environmental changes.

It has been suggested that complex food webs and communities with high biodiversity are stable and not highly prone to disturbances (see Clements & Newman 2002, Begon et al. 2005). This suggests that soil food webs that have high structural complexity and diversity are also stable. The concept of stability is built upon two aspects: resistance and resilience. Resistance can be defined as the capacity of the system, including populations and communities, to resist disturbance while resilience is the capacity of, or speed with which a system recovers to its former state after a disturbance (Seybold et al. 1999, Begon et al. 2005, Clements & Rohr 2009).

In theory, the recovery of a community or ecosystem to its pre-disturbed state is not always likely to occur (see Matthews et al. 1996, Van Straalen 2003, Clements & Rohr 2009). Furthermore, despite its common usage in the literature, no universally accepted definition for the concept "recovery" exists. The rate of recovery appears to be context-dependent and different components of the community and ecosystem may recover at different rates (Clements & Newman 2002). Thus, the recovery of communities back to a pre-disturbed state does not necessarily mean recovery of ecological functions of the system – or vice versa. As a consequence, when recovery of the entire system is the goal, both structural and functional indicators should be assessed (Clements & Newman 2002), as is done in this thesis. Here, studying the potential recovery of the boreal forest ecosystem from lead-derived

disturbances was possible due to the location of a reference site in the same forest stand as the disturbed sites. In many cases, a lack of such a reference system limits assessing and even defining recovery (Clements & Newman 2002).

Although the recovery of soil communities after a disturbance has received a fair amount of interest (e.g. Liiri et al. 2002a, Lindberg & Bengtsson 2006, Frouz et al. 2008), little is known about the recovery of soil communities from toxicant-derived disturbances (see Filser 2008). Furthermore, the recovery of ecosystems at metal-contaminated sites is somewhat complicated due to the persistent nature of metals: unlike degradable organic compounds, metals are not likely to disappear quickly from the system.

1.3 Shooting ranges, lead and the environment – the scope of the problem

Lead (Pb) is a heavy metal with no known biological function and is thus nonessential to all organism, unlike some other metals like copper (Cu) or zinc (Zn) (White et al. 2007). Instead, Pb is known to be toxic, e.g. by replacing calcium (Ca) in its actions and by interacting with proteins in various tissues; the nervous system being the most sensitive target in vertebrates (Stohs & Bagchi 1995, Pattee & Pain 2002, White et al. 2007). However, due to its suitable characteristics for shooting purposes, Pb is commonly used in bullets and shotgun pellets. Despite their toxicity (Mateo et al. 2001, Pattee & Pain 2002, Pokras & Kneeland 2008), shotgun pellets containing 94 – 99 % of Pb (Takamatsu et al. 2010) are used at clay pigeon shooting ranges and Pb-contaminated soils at shooting range areas are found around the world (Darling & Thomas 2003, Sorvari et al. 2006, Rooney et al. 2007, Luo et al. 2014).

After being shot, pellets spread over substantially wide areas and enter nearby ecosystems, which in Finland are typically coniferous forest sites (Sorvari et al. 2006). In the soil, pellets start to corrode and elemental Pb to oxidize due to the effects of soil water, oxygen (O_2) and carbon dioxide (CO_2) (Lin et al. 1995, Cao et al. 2003a, Scheetz & Rimstidt 2009, Takamatsu et al. 2010). Some of the Pb fractions can be transformed into more mobile and bioavailable forms and be leached down in the soil profile or accumulated in the biota (Hui 2002, Labare et al. 2004, Robinson et al. 2008).

Leaching of Pb through the soil can further pose risks to ground water quality (Sorvari 2007). Indeed, shooting ranges in Finland are typically located on sandy, well-drained eskers susceptible to leaching and one-third of all shooting ranges are situated close to aquifers supporting groundwater reservoirs (Sorvari et al. 2006).

Risks of Pb contamination to ecosystems close to shooting ranges are evident. For example, elevated tissue Pb concentrations at shooting range areas have been reported in plants (Hui 2002, Labare et al. 2004, Robinson et al. 2008), invertebrates (Vyas et al. 2000, Hui 2002, Labare et al. 2004, Migliorini et al. 2004, Reid & Watson 2005), frogs (Stansley & Roscoe 1996), birds (Vyas et al. 2000, Lewis et al. 2001) and

mammals (Ma 1989, Stansley & Roscoe 1996, Lewis et al. 2001). Some studies at shooting ranges have also reported Pb-induced changes in soil microbial (Rantalainen et al. 2006, Hui et al. 2009, Hui et al. 2011) and faunal (Migliorini et al. 2005) communities.

Lead-induced changes in these crucial components of the soil food web may further be reflected in the ecosystem due to the crucial role of the soil biota in various soil processes, including nutrient cycling (Setälä 1995, Heneghan & Bolger 1998, Laakso & Setälä 1999, Bardgett & Wardle 2010). However, the effects of shooting-derived Pb at the ecosystem level are poorly known (Rantalainen et al. 2006), even though more than 2000 shooting ranges are estimated to exist in Finland alone (Naumanen et al. 2002, Sorvari et al. 2006). Even less is known about long-term changes in the fate and effects of Pb in these contaminated ecosystems after cessation of the shooting activity. This is surprising given that more than half of the shooting ranges in Finland have already been abandoned (Sorvari et al. 2006).

In the long-term, bioaccumulation, leaching and overall toxic effects of Pb in the ecosystem nearby shotgun shooting ranges can be expected to increase due to the gradual release of Pb from the pellets (Lin et al. 1995, Cao et al. 2003a, Scheetz & Rimstidt 2009, Takamatsu et al. 2010). However, in boreal forests with mor-type soils, the formation of a less-contaminated soil layer on top of the soil may reduce the toxic effects due to litter accumulation on top of the contaminated soil. It is possible that this less-contaminated surface soil could promote the recovery of the ecosystem by providing less-contaminated habitat for the soil community.

2. OBJECTIVES

In this thesis, ecosystem-level research is presented in which the fate and effects of Pb in a boreal forest ecosystem was explored. Furthermore, special emphasis was placed on assessing whether the ecosystem can recover after range abandonment. These issues were possible to study by having three study sites in the same pine forest stand that differed in contamination history: an uncontaminated control site together with an active and an abandoned shotgun shooting range. Lead pellet loads and the duration of the shooting activity at the shooting range sites were similar, but shooting at the abandoned range had ceased 20 years ago, when the shooting activity was relocated to the recently active shooting range. The main objective of the thesis was to shed light on the following questions:

1. What is the fate of shooting-derived Pb in a boreal forest ecosystem? (I)
2. What are the effects of Pb on the food web of boreal forest soils? (II, III)
3. Do potential Pb-derived changes in the soil biota result in alterations in ecosystem processes? (III, IV)
4. Do the fate and effects of Pb in boreal forest ecosystems change in the long run and can the ecosystem recover after range abandonment? (I, II, III, IV)

3. MATERIAL AND METHODS

3.1 Study sites

The study sites were located in a pine forest stand at the Hälvälä shooting area (61º00'N, 25º29'E), Southern Finland. The area is situated on an end moraine of the latest glaciation and belongs to a first class ground water area. Three sites were studied at this area:

1. Active shooting range (New Contaminated site [NC]), where shooting has occurred since 1987
2. Abandoned shooting range (Old Contaminated site [OC]), with shooting activity during 1964 - 1986
3. Control site (Control) without shooting activities in its history

At the two contaminated sites, clay pigeon shooting with shotguns had been practised for approximately the same period of time, but it occurred 20 years earlier at OC. Both of these study sites were established in areas where the pellets fall after shooting. Ten study plots of 1.5 m x 1.5 m in size were established at each site, with plots at the two contaminated sites placed along a Pb concentration gradient (Fig. 1).

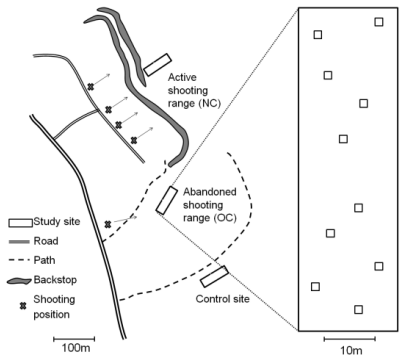

Figure 1. Location of the three study sites – a control site and the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges at the Hälvälä shooting range area. Ten plots per study site were established, as illustrated in the rectangle to the right (Selonen et al. 2012).

The three study sites were located 200 – 500 m apart (Fig. 1) in the same forest stand, which regenerated naturally in 1980s with Scots pine (*Pinus sylvestris*) as the dominant tree species. Shrubs *Vaccinium vitis-idaea* and *V. myrtillus* and grasses *Deschampsia flexuosa* and *Calamagrostis arundinacea* dominate the field layer and mosses *Pleurozium schreberi* and *Dicranum* sp. the bottom layer of the forest (Rantalainen et al. 2006).

Soil at the sites is stony moraine with weak podsolization (Hartikainen & Kerko 2009) and with a mor-type organic soil horizon. The organic soil horizon can be divided into three layers (see Binkley & Fisher 2013): uppermost litter layer (OL / Oi), below which is a 3 – 6 cm thick fermentation (F) layer (OF / Oe) with partly decomposed plant residues and the lowest, approximately 1 cm thick humus (H) layer (OH / Oa) with well-decomposed, more homogeneously structured material. When soils at the sites were assessed, moss and litter were removed so that only the F and H layers were included in the analyses.

3.2 Sampling

Soil sampling (I, II, III, IV)

The organic soil horizon was sampled from two locations at each plot in May 2005 (Spring I), October 2005 (Autumn) and in May 2006 (Spring II), except in Spring II when only five plots were

sampled from one location at the Control. At each location, four samples were taken from the entire organic soil horizon using a 22 cm^2 steel auger: i) a sample for the analysis of soil Pb concentrations, ii) a sample for basal respiration and substrate induced respiration (SIR) measurements, and samples to extract iii) microarthropods (Arthropoda) and iv) enchytraeid worms (Oligochaeta, Enchytraeidae). Nematodes (Nematoda) were sampled from the entire organic soil horizon in Spring II using a steel auger 3.6 cm in diameter.

In Spring II all the samples mentioned above, except the samples for basal respiration and SIR measurements, were divided into two layers: upper fermentation (F) layer and lower humus (H) layer (Table 1). Samples for the analysis of Pb were already divided this way in Autumn (Table 1).

In addition, a combined sample from the H layer was taken at each sampling location. From these samples, nematodes, protozoans (Protozoa) and phospholipid fatty acids (PLFA; to estimate soil fungal and bacterial biomass) were extracted (see Table 1). Soil organic matter (SOM) content, moisture and pH, as well as nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) were also analysed from these samples. In addition, soil Pb was analysed from this sample in Spring I. PLFA analyses were only performed from one sample per plot.

The total mass of Pb pellets per plot was estimated by weighing the pellets of six soil samples taken from each study plot at NC and OC. These soil samples were first used for microarthropod extractions.

In addition, three 30 cm x 30 cm samples of the organic soil horizon per study site were taken in Autumn to extract microarthropods for Pb analyses. At NC and OC, these samples were taken from the most contaminated parts of the sites.

Lysimeters (I, III)

To measure Pb and nutrient (NO_3^-, NH_4^+, PO_4^{3-}) leaching from the organic soils, five zero-tension pan lysimeters were installed beneath the organic soil horizon at the most contaminated parts at NC and OC and at the control site in June 2005. The lysimeters had an area of 35 cm x 25 cm and height of 8 cm. Water leachates from each lysimeter were collected in August and October 2005 and in May, June, August and October 2006. The samples were filtrated (0.45 µm polyethersulfone filters, VWR) and the acidity of the samples for Pb analyses was adjusted to 1 % (v/v) by adding HNO_3.

Trees and understorey vegetation (I, IV)

The growth of Scots pines (*P. sylvestris*) was assessed from 12 individual trees per site by measuring the width of annual tree rings drilled about 15 cm aboveground into the tree trunks in 2005. At the two contaminated sites, pines growing close to the most contaminated plots were chosen. Needle samples for nutrient and Pb analyses, consisting of three-year old or younger needles, were taken from seven of these trees at each study site in August 2007.

Lead concentrations were also measured from the dominant understorey

plant species and from fungal sporocarps. For this, leaves of the grass *C. arundinacea* and of blueberry *V. myrtillus* were collected from each study plot in August 2005, and sporocarps of *Amanita muscaria* and *Lactarius rufus* in August – September 2005.

Litter traps (I, IV)

To measure litter input, two 0.08 m^2 litter traps were placed next to each study plot, 80 cm aboveground. The traps were emptied every month from July to October 2005 and again after winter from May to October 2006. Litter was dried at 60 ºC for three days before measuring the dry mass.

In addition to litter, Pb pellets accumulated in the litter traps at NC and enabled to estimate the monthly accumulation of Pb pellets at the site.

3.3 Laboratory measurements

Soil fauna (I, II, IV)

Enchytraeid worms and nematodes were extracted from the soil samples using wet funnels (O'Connor 1962) and microarthropods using a high gradient extraction method (Macfadyen 1961). After extraction, the fauna were counted and microarthropods and nematodes fixed in 70 % ethanol. Collembola and Mesostigmata were identified to family level, Oribatida to superfamily level and Nematoda mostly to family level and separated into feeding guilds (Yeates 1971) (Table 1). The biomass of enchytraeid worms (mostly *Cognettia sphagnetorum*, Vejd.) was calculated according to the equation in Abrahamsen (1973).

After counting, enchytraeid worms from the Autumn sampling event were transferred to Petri dishes with a small amount of boiled and cooled tap water. After a few days in a cold room – as soon as the intestines were empty – the worms were dried at 70 °C and individuals per sample were pooled for Pb analysis. Accordingly, microarthropods extracted from the three 30 cm x 30 cm samples per site were divided into groups of Oribatida, Mesostigmata and Collembola, transferred onto moistened filter paper for five days to empty their guts and then

Table 1. Soil samples taken during the three sampling events (Spring I, Autumn and Spring II) from the fermentation layer (F), the humus layer (H) or from the entire organic soil horizon (F + H), and the articles in which these data are used.

Sample	Articles	Spring I	Autumn	Spring II
Pb pellet load	I	(F + H)	(F + H)	(F + H)
Soil Pb	I, II, III, IV	H	F, H	F, H
Soil pH, moisture, SOM	I, II, III, IV	H	H	H
Soil nutrients	III, IV	H	H	H
PLFA	III, IV		H	H
Enchytraeid worms	I[a], II, IV	F + H	(F + H)[a]	F, H
Microarthropods	I[a], II, IV	(F + H)[b]	(F + H)[b]	F[b], H[b]
Nematodes	II, IV	H[b]	H[b]	F, H
Protozoans	II, IV		H	H

[a] Pb concentrations measured

[b] Identified to family or superfamily level

dried at 70 °C before Pb analyses.

Protozoans were extracted using the method in Ekelund (1998). Five grams of soil was suspended in 100 ml of 0.01 % (m/m) Trypticase Soy Broth (TSB), after which a dilution series of the suspensions were prepared on 96-well microtitre plates. The presence of protozoans in the wells was checked using a microscope, 7 and 21 days after extraction, and the number of protozoans was estimated using the most probable number (MPN) method with Microsoft Excel software modifying the method of Briones and Reichardt (1999).

Pb analyses (I, II, III, IV)

To extract Pb fractions from the soil, Pb samples were divided into three subsamples and pellets carefully removed from the soil. Total Pb (tot-Pb) was extracted with 65 % HNO_3 in an autoclave for 30 min. Available fractions of Pb were estimated using H_2O (H_2O-Pb) and 0.01 M $CaCl_2$ ($CaCl_2$-Pb) extractions by shaking 5 g of fresh soil with 50 ml of either distilled water or a 0.01 M $CaCl_2$ solution at 250 rpm for 2 h (Houba et al. 1996, Smit et al. 1997). The soil extracts and water leachate samples were analysed using a flame or graphite furnace atomic absorption spectrometer (AAS; Thermo M series).

Pine needle, plant leaf and sporocarp samples were dried at 50 ºC for 4 to 5 days, homogenized and extracted with 1:5 H_2O_2 (30 %) : HNO_3 (65 %) using microwave digestion (Milestone, MLS-1200 MEGA). The extracts were analysed for Pb by an inductively coupled plasma mass spectrometer (ICP-MS; Perkin-Elmer Elan 6000).

The enchytraeid worm samples were extracted using ultrasound assisted extraction (Väisänen et al. 2002), and Pb concentrations in the samples were determined by GFAAS at the Department of Chemistry, University of Jyväskylä, Finland. Pb concentrations in Oribatida and Mesostigmata were determined as solid samples by GFAAS (Analytic Jena, AAS Jena Zeenit 60 with Solid Sampling System SSA 61Z) at KCL Kymen Laboratorio Ltd., Finland. Due to the low biomass of Collembola in the samples, their Pb concentrations could not be analysed.

Nutrient analyses (III, IV)

For the NO_3^- and NH_4^+ analyses, 5 g of humus samples were extracted in 50 ml of 1 M KCl for 4 h. Phosphate (PO_4^{3-}) was similarly extracted but distilled H_2O was used as a solvent. After filtration (Whatman 589/3 and 0.45 µm polyethersulfone filters, VWR), soil extracts and water leachates collected from the lysimeters were analysed using a Lachat QuickChem 8000 FIA analyser.

Nutrient contents (N, P, K, Ca, Mg, Mn, Zn, B and Cu) of the pine needles were measured at the Finnish Forest Research Institute (Metla).

Moisture, pH and SOM (I, II, III, IV)

For pH measurements, 15 ml of soil was shaken in 75 ml of distilled H_2O (Spring I and Autumn) or 0.01 M $CaCl_2$ (Spring II) for 5 min. After 18-20 h, the suspensions were shaken briefly, after which pH was measured. Soil moisture was analysed as mass loss after drying samples at 105 ºC for 24 h and soil

organic matter (SOM) after ignition at 550 ºC for 4 h.

PLFA analysis (III, IV)

To explore the microbial community composition in the H layer, phospholipid fatty acid (PLFA) profiles of the soils were determined. PLFAs are components of cellular membranes of organisms that decompose quickly after the organism dies (Bååth et al. 1998, Kaur et al. 2005). Although PLFAs are not species-specific and thus do not give precise information of the species, they are considered a useful tool for environment monitoring, indicating both shifts in the microbial community composition (Frostegård et al. 1993, Bååth et al. 1998, Kaur et al. 2005) and phenotypic changes in the PLFA composition in cellular membranes due to disturbance or stress (Kaur et al. 2005, Frostegård et al. 2011).

Soil samples for PLFA analysis were freeze-dried and stored at -80 ºC until analysed. Two subsamples (0.5 g and 1.0 g) were taken from each sample for PLFA extractions, which were conducted using the method by Bligh and Dyer (1959) with modifications by White et al. (1979). After extraction, samples were analysed using gas chromatography-mass spectrometry (Shimadzu GC-MS QP5000), with fatty acids 13:0 and 19:0 as internal standards. The PLFA 18:2ω6,9 was used to indicate soil fungi (Frostegård & Bååth 1996), and the sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, cy17:0, 17:00, 18:1ω7 and cy19:0 indicated bacteria (Frostegård & Bååth 1996). Using these concentrations [nmol g^{-1} (dry mass (dm))], the fungal/bacterial ratio was calculated for each sample. In addition, ratios of cyclopropyl fatty acids and their precursors (cy17:0/16:1ω7c and cy19:0/18:1ω7c) were calculated, since they have been found to indicate stress (Bossio & Scow 1998, Zhang et al. 2006). In addition, the total amount of PLFA was used as an estimate for total microbial biomass.

Basal Respiration and SIR (IV)

Soil microbial activity was estimated by basal respiration and the potentially active microbial biomass by the substrate induced respiration (SIR) method (Anderson & Domsch 1978). To avoid the release of Pb from the pellets, intact samples of the organic soil horizon were placed carefully in 200 ml plastic jars with lids. Both basal respiration and SIR were estimated by analysing the production of CO_2 after 2 h incubation. In both analyses, a 1 ml air sample was taken from each jar before and after 2 h incubation. CO_2 concentrations were analysed using the Easy Quant Universal Carbon analyser. For the SIR analysis, a glucose solution (glucose 2 % of soil fresh mass) was added 2 h before the first measurement.

3.4 Litter bag experiment (IV)

The rate of litter decomposition was studied using the litterbag approach (Swift & Anderson 1989). Pine (*P. sylvestris*) needle litter was collected from an uncontaminated forest and fresh grass (*C. arundinacea*) leaves from each study site (NC-grass, OC-grass and C-grass). The litter was air-dried and Pb concentrations in three 0.5 g subsamples

from each litter type were measured. Lead concentrations (mean [µg g^{-1} (dm)] ± SE) were 4.8 ± 1.3 in the pine needle litter, 5.2 ± 0.4 in the C-grass, 27.6 ± 3.2 in the NC-grass and 21.0 ± 0.5 in the OC-grass.

One gram of each grass litter type and 2.5 g of pine needle litter were separately placed into nylon mesh bags of size 8 x 8 cm^2 and mesh size of 1 mm, allowing micro- and mesofauna to enter the litterbags. One bag of each litter type was buried under the moss and litter at each study plot in June 2005. To estimate litter decomposition rates, litter mass loss was measured after 18 months.

3.5 Statistical analyses

Data from study plots, pine needles and the litter bag experiment (I, II, III, IV)

Before statistical analyses, data of some variables were log_{10} or square-root transformed to meet the requirements of parametric tests. If the requirements were not met after transformation, non-parametric tests were used. In addition, obvious outliers in the Pb data (13 out of 630 extractions, likely due to Pb pellet particles accidentally remained in the samples) were identified by Normal Q-Q plots created in the statistical software package PASW 18 and removed before statistical analyses (I – IV).

To compare the availability of Pb between NC and OC, proportions of $CaCl_2$-Pb and H_2O-Pb of tot-Pb were calculated (I). To compare the vertical distribution of Pb and faunal abundances between NC and OC, the relative proportion of the Pb fractions (I) and soil faunal groups (II) between the F and H layer (F/H –ratio) was calculated for each sample.

Average values of each study plot (n = 10) at each sampling event were considered replicates in the statistical analyses, despite the fact that they may not represent independent sampling units. Since temporal variation of the measured variables between sampling events was beyond the scope of this study, each sampling event was treated separately in the statistical analyses (I – IV).

Differences between the study sites (Control, NC, OC) were analysed using one-way ANOVA followed by Tukey's test, or by using nonparametric Kruskal-Wallis test followed by pairwise comparisons with Mann-Whitney test (I – IV). Grass litter decomposition was analysed using two-way ANOVA to reveal differences between the study sites and between the origins of the litter (IV).

Differences in the concentrations of different Pb fractions (I) and soil faunal abundances (II) between the F and H layers within sites were analysed using paired sample t-tests or non-parametiric Wilcoxon signed-rank test for two related samples.

Pearson's correlation analysis, or in the case of non-parametric data, Spearman's correlation analysis, was used to analyse relationships between Pb availability, Pb accumulation and soil Pb concentrations in different soil layers within the two contaminated sites (I). Stepwise multiple regression analyses were used to reveal possible relations between biological data and soil properties at the contaminated sites, using soil moisture, SOM, pH and one of

the analysed Pb fractions at a time as independent variables (II, III). Regression analyses were applied to explore the responses of soil nutrients and pH to soil Pb fractions (III). PASW Statistics version 18.0 (SPSS Inc.) was used to perform the analyses.

Lysimeter and litter trap data (I, III, IV)

Seasonal and between-site differences in Pb (I) and nutrient (III) concentrations in leachate waters and the litter fall (IV) (log_{10}-transformed when necessary) were analysed using repeated measures ANOVA followed by multiple comparisons (Tukey's test). Study site was used as a between-subject factor and sampling as a within-subject factor. Analyses were run with PASW Statistics version 18.0 (SPSS Inc.).

Community analyses (II, III, IV)

All community analyses were performed using PC-Ord 6.0 (MjM Software Design). To visualize differences in microarthropod and nematode communities between the study sites (II), non-metric multidimensional scaling (NMDS), based on Sorensen's distance measure, was used. To show the potential relations between taxa and the measured environmental parameters (tot-Pb, $CaCl_2$-Pb, H_2O-Pb, SOM, moisture and pH), a joint plot with Pearson correlation (r_p) and a significance level of 0.05 as a criterion was used. Differences between the study sites with respect of community structures were tested using the PerMANOVA test (Anderson 2001) followed by pairwise comparisons. In addition, typical microarthropod and nematode taxa for each study site were identified using the Indicator Species Analysis (Dufrene & Legendre 1997).

Redundancy analysis (RDA) (Legendre & Legendre 1998) was used to visualize differences in microbial communities (III) and in structural and functional variables (IV) between study plots at the different study sites, and the response of these variables to soil properties (tot-Pb, pH, moisture, SOM, NO_3^-, NH_4^+ and PO_4^{3-}). In RDA of microbial communities (III), relative concentrations (% of the total PLFA) in the sample were used as response variables. In RDAs of the structural and functional data (IV), soil faunal groups, basal respiration, SIR, decomposition of different litter types, and in the H layer also fungi, bacteria and protozoans were used as response variables. In the latter case (IV), two RDAs were performed – one including structural data of the F layer and one of the H layer. Before the RDAs were performed, PLFA data (III) were centred and functional and structural data (IV) were centred and standardized to its variance, since the variables were in different measurement units. Multi-Response Permutation Procedure (MRPP) (Mielke et al. 1976) was performed to test for differences between the study sites as regards to their PLFA profile (III) and structural and functional attributes of the soil food web (IV).

4. RESULTS AND DISCUSSION

4.1 The fate of lead (I)

Lead in the soil

Lead pellet load at the two contaminated sites was extremely high, reaching up to 4 kg m^{-2}. Furthermore, tot-Pb concentrations after the removal of pellets were very high at the contaminated sites (Table 2). Consequently, Pb concentrations at each sampling plot easily exceeded the ecological risk guideline value of 750 mg kg^{-1} of the Finnish legislation, set for areas for use other than settlements or recreation (Government decree on the assessment of soil contamination and remediation needs 214/2007). The highest tot-Pb concentrations, over 50 000 mg kg^{-1}, were found in the humus (H) layer at OC.

Even though Pb pellet loads at the active (NC) and abandoned (OC) shooting ranges were similar, a remarkable difference in the vertical distribution of Pb in the organic soil horizon was found. Whilst the highest tot-Pb concentrations were found in the H layer at OC, Pb concentrations were twice as high in the F layer at NC as at OC (Table 2). Thus, the F/H-ratio for tot-Pb, as well as that for $CaCl_2$-Pb was higher at NC than at OC (Table 2, Fig. 2). This indicates that litter accumulation on the soil surface and the subsidence of pellets in the F layer due to gravity have led to decreased tot-Pb concentrations in the topmost soil layer. At the same time, the gradual release of Pb from corroding pellets (Levonmäki et al. 2006, Takamatsu et al. 2010) has increased Pb

Table 2. Soil Pb concentrations (mg kg^{-1}; mean ± SE; n = 10) and vertical distribution of Pb at the uncontaminated control site, the active shooting range (NC) and the abandoned shooting range (OC). Total Pb, H_2O extractable Pb and 0.01 M $CaCl_2$ extractable Pb concentrations (mg kg^{-1}(dm)) in the fermentation (F) and humus (H) layer, and the ratio of Pb concentration in the F layer to humus layer (F / H) are shown. Differences between sites are tested by Kruskal-Wallis test, followed by Mann-Whitney U Test. Within each row, sites with different letters in brackets were significantly different at the 0.05 level (Selonen et al. 2012).

	Control	NC	OC
Total Pb			
F layer	44 ± 8 (a)	23 175 ± 3 175 (b)	12 239 ± 1 596 (c)
H layer	125 ± 18 (a)	19 034 ± 3 469 (b)	28 328 ± 4 511 (b)
F / H	0.35 ± 0.06 (a)	1.74 ± 0.22 (b)	0.52 ± 0.05 (c)
H_2O extractable Pb			
F layer	0.04 ± 0.01 (a)	42 ± 7 (b)	57 ± 12 (b)
H layer	0.09 ± 0.01 (a)	17 ± 4 (b)	38 ± 6 (c)
F / H	0.75 ± 0.22 (a)	4.48 ± 0.79 (b)	2.54 ± 0.57 (b)
$CaCl_2$ extractable Pb			
F layer	0.55 ± 0.05 (a)	867 ± 173 (b)	513 ± 93 (b)
H layer	1.73 ± 0.22 (a)	887 ± 179 (b)	1 399 ± 267 (b)
F / H	0.46 ± 0.07 (a)	1.90 ± 0.39 (b)	0.80 ± 0.34 (c)

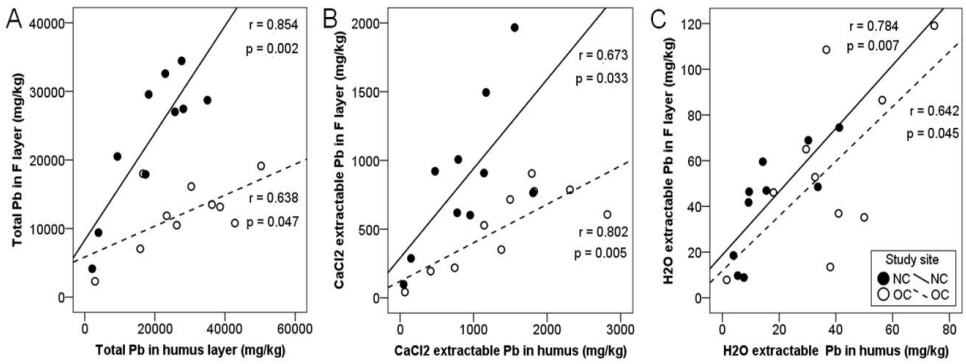

Figure 2. Vertical distribution of total Pb (a), 0.01 M $CaCl_2$ extractable Pb (b) and H_2O extractable Pb at the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges. Relationships of Pb concentrations between the fermentation (F) (*y*-axes) and humus (H) layers (*x*-axes) are shown. In addition, Pearson correlation coefficients and their significance are shown (Selonen et al. 2012).

concentrations deeper in the soil. These processes have led to a gradual change in the vertical distribution of Pb in the soil. This is due to the slow turnover of organic material in mor-type boreal forest soils (DeAngelis 2009) and by the low intensity of soil mixing due to the scarcity of earthworms that can transport material – including contaminants – to the soil surface (Haimi 2000, Eijsackers et al. 2008). The consequences of changes in the vertical distribution of Pb are discussed further in sections 4.6 and 4.7 below.

Although tot-Pb concentrations were high at the contaminated sites, it is notable that only part of it was bioavailable or mobile. As the estimates for available Pb (Houba et al. 1996, Seguin et al. 2004), less than 0.5 % of tot-Pb was found extractable in H_2O, and at maximum 7 % in 0.01 M $CaCl_2$ (Table 2). In these mor-type soils, the adsorption and complexation of Pb with solid organic matter are likely to bind Pb tightly in the organic soil. On the other hand, low pH of these soils can promote the mobility and bioavailability of Pb (McBride et al. 1997, Sauve et al. 1998, Pattee & Pain 2002, Luo et al. 2014). Either way, Pb was mobilised to the extent that part of it was detected in water leachates at both shooting range sites (figure 6 in I), exceeding both the WHO recommended value of 10 µg l^{-1} and the EU statutory limit of 50 µg l^{-1} for drinking water. In addition, elevated Pb concentrations in plants and the soil fauna were found at both shooting range sites, in accordance with other shooting range studies (Cao et al. 2003a, Cao et al. 2003b, Labare et al. 2004). This indicates that despite relatively low availability, Pb loads at these sites are high enough to enter the soil food web and the aboveground biota, as discussed below.

Lead in the biota

Concentrations of Pb in the biota were generally higher at the two contaminated sites than at the control site, indicating that Pb bioaccumulates and transports at different trophic levels in the food web (table 3 in I). Highest Pb concentrations, reaching over 1 000 µg g^{-1} (dm) at NC, were found in enchytraeid worms, which

are soft-bodied, detritivorous organisms that are exposed to soil contaminants both orally and dermally (Didden & Römbke 2001). High Pb concentrations were also found in other soil faunal groups; oribatid mites (Oribatida) and mesostigmatid mites (Mesostigmata) (table 3 in I). However, oribatid mites had higher concentrations of Pb than predatory mesostigmatid mites (table 3 in I), despite their lower trophic position in the food web. Hågvar and Abrahamsen (1990) and Migliorini et al. (2005) suggested that oribatid mites may be exposed to contaminants to a greater extent than predators by ingesting soil and fungal hyphae that accumulate Pb efficiently.

Tissue concentrations of Pb in fungal sporocarps and leaves of plants were substantially lower than in the soil fauna (table 3 in I), but were similar to vascular plants in other shooting range studies (Manninen & Tanskanen 1993, Rooney et al. 1999, Hui 2002, Labare et al. 2004). However, the relatively low concentrations of Pb in leaves do not necessarily mean that toxic effects of Pb do not exist in plants, since the transport of Pb from roots to foliar parts is usually limited and Pb concentrations in the roots tend to be higher than in leaves (Rooney et al. 1999, Pattee & Pain 2002). The highest concentrations, on average 30 μg g^{-1} (dm) at both contaminated sites, were found in leaves of the grass *C. arundinacea*. Lead concentrations in these leaves, as well as in blueberry (*V. myrtillus*) leaves increased linearly with increasing tot-Pb in the soil (figure 5 in I). In fungal sprocarps and the soil fauna, such a clear relationship was not found, suggesting more complex or varying toxicokinetics and handling of Pb in these organisms as compared to plants (see Spurgeon et al. 2011).

Although H_2O and 0.01 M $CaCl_2$ extractions were used to estimate available Pb, the concentrations of Pb in plant leaves correlated more strongly with tot-Pb than with H_2O-Pb and $CaCl_2$-Pb. This is in line with findings by Labare et al. (2004) from another shooting range area. However, overall Pb concentrations in plants were at the same level with H_2O-Pb in soil and in soil fauna at the same level with $CaCl_2$-Pb. The weaker correlation may arise from substantial fluctuations of these fractions of Pb in the soil. Thus, it seems that the actual bioavailable Pb fraction correlates better with tot-Pb, whilst the $CaCl_2$-Pb and H_2O-Pb fractions are better estimates for the overall level of bioavailable Pb. Because the abundances of many biotic groups investigated correlated better with tot-Pb than with the other Pb fractions, it further suggests that tot-Pb correlates with actual bioavailable Pb concentrations in the soil (II, III).

4.2 Effects of Pb on soil pH (III)

Shooting-derived Pb affects not only organisms but also the abiotic environment by increasing soil pH. Soil pH at the contaminated sites was higher than at the control site during both spring seasons (figure 1 in III) and increased with increasing Pb concentrations in the soil (Fig. 3). An increase in pH can be explained by the oxidation of elemental Pb, when pellets corrode in the soil [$Pb_{(s)} + 0.5O_2 + 2H^+ = Pb^{2+} + H_2O$] (Rooney et al. 2007, Scheetz & Rimstidt 2009).

Since different soil biota have different pH optima, an increase in pH can induce changes in the composition of the soil food web (Ingham et al. 1989, Alphei et al. 1996, Fierer et al. 2009). Increased pH may also decrease the bioavailability and toxicity of Pb (Bradham et al. 2006, Giller et al. 2009, Kools et al. 2009, Luo et al. 2014). Indeed, as reported by Giller et al. (2009), direct effects of metals on the biota are difficult to separate from indirect effects related to pH changes (see also later sections).

It is also notable that, contrary to solid metals at e.g. shooting ranges, contamination with metal salts or cations may decrease soil pH (Frostegård et al. 1993, Speir et al. 1999, Dai et al. 2004) and thus affect the biota and bioavailability of the metal oppositely. Thus, due to the interactions between different forms of metals, soil pH and the soil biota, the effects of metals on soil pH should be acknowledged when evaluating the effects of metals in soils.

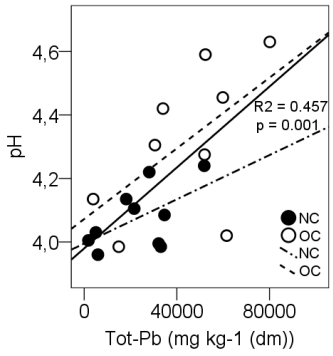

Figure 3. Relationship between pH and total Pb in humus of the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges in Spring II (Paper III).

4.3 Effects of Pb on the soil food web

Soil faunal communities (II)

Soil faunal communities were clearly affected by shooting-derived Pb, seen both as decreased abundances and as changed community compositions. Generally, differences in faunal abundances between the shooting range sites and the control site were most pronounced in Autumn, when abundances at the control site were substantially higher than in Spring I and II, whilst abundances at the contaminated sites remained rather low, even in Autumn (Fig. 4). This suggests a decreased ability of the fauna to recover after winter at the contaminated sites, which, in turn, indicates a decreased resilience of the populations, making them vulnerable to additional stress (Clements & Newman 2002).

Likely the most striking finding concerning the soil fauna was the total absence of enchytraeid worms in the H layer at OC, where the highest Pb concentrations were found. Enchytraeid worms were also negatively affected by Pb in the F layer (figure 3 in II) and when the whole organic soil layer was considered (Fig. 4, figure 4 in II). When compared to the other faunal groups – nematodes, microarthropods and protozoans – enchytraeids was the only group affected negatively at each sampling event and at both contaminated sites (Fig. 4). Enchytraeid worms are considered sensitive to metals in other studies as well (Salminen et al. 2001b, Salminen et al. 2001a, Haimi & Mätäsniemi 2002, Kools et al. 2009). A decline in the abundance of enchytraeid

worms, consisting mostly of one species (*C. sphagnetorum*) in the current study, may affect decomposition processes and nutrient cycling negatively (see also Salminen et al. 2001b and section 4.4) because of the important role of *C. sphagnetorum* as a keystone species in boreal forest soils (Huhta et al. 1998, Laakso & Setälä 1999).

Within the nematode community the negative effects of Pb were strongest in omnivores. Omnivorous and predatory nematodes are sensitive to heavy metals due to their long generation times, low reproductive rates and permeable cuticles (Korthals et al. 1996, Bongers et al. 2001, Shao et al. 2008). The effects of Pb on nematodes were also reflected in the community composition, especially in Autumn, where communities at both contaminated sites differed clearly from the community at the control site (figure 5 in II).

Microarthropods were also affected by Pb (Fig. 4, figures 3 & 6 in II), with Phthiracaroidea (Oribatida) showing the highest sensitivity at family level (table 1 in II). In contrast, some groups were positively related to soil Pb (Online Resources 1, 2 & 3 in II), especially Galumnoidea (Oribatida), which was identified as typical species of Pb-contaminated soils using the Indicator Species Analysis. Since some microarthropod groups decreased while others increased due to soil Pb contamination, Pb also affected microarthropod community composition, in accordance with a study at another shooting range site (Migliorini et al. 2005). Variation in the susceptibility of different soil microarthropods to heavy metals can lead to a lack of responses at higher taxonomic levels (Migliorini et al. 2005, Khalil et al. 2009). However, in this study, the total number of microarthropods also decreased with Pb, in accordance with some other studies at metal-contaminated sites (Haimi & Siira-Pietikäinen 1996, Haimi & Mätäsniemi 2002).

Even though the positive responses of some microarthropod groups and protozoans to soil Pb seem surprising, numerous studies have also reported similar relationships in metal-

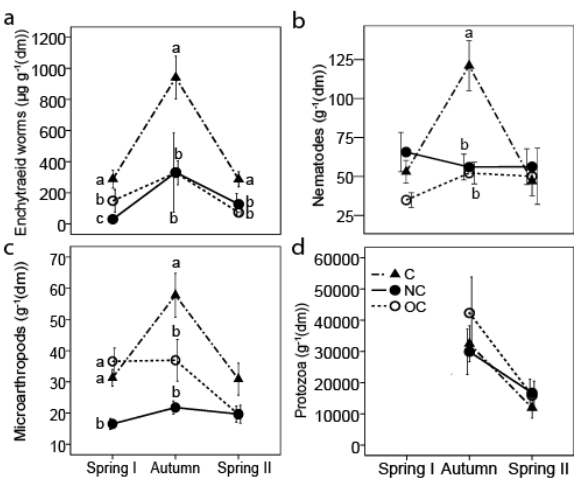

Figure 4. Abundance of enchytraeid worms (a) nematodes (b), microarthropods (c) and protozoans (d) (mean ± SE) at the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges and the control site (C). Enchytraeid worms (a) and microarthropods (c) were sampled from the entire organic soil layer (F + H), while nematodes (b) and protozoans (d) were sampled from the H layer. Within each sampling event, sites with different letters were significantly different at the 0.05 level (Selonen et al. 2014).

contaminated soils (Haimi & Siira-Pietikäinen 1996, Salminen et al. 2001a, Zaitsev & van Straalen 2001, Georgieva et al. 2002, Migliorini et al. 2004, Khalil et al. 2009). These relations likely result from indirect effects of Pb due to changes in the biotic or abiotic environment (see Didden & Römbke 2001, Georgieva et al. 2002, Rohr et al. 2006, Clements & Rohr 2009). Species that tolerate the contaminant better than others may benefit from soil contamination by gaining a competitive advantage (Salminen et al. 2001a, Georgieva et al. 2002). They may also profit from decreased predation pressures (Georgieva et al. 2002), increased food resources (Salminen et al. 2001a), or contaminant-induced changes in soil properties (Russell & Alberti 1998). For instance, increased soil pH (see section 4.2 above) may affect the soil biota by decreasing the toxicity and bioavailability of Pb (Bradham et al. 2006, Kools et al. 2009, Luo et al. 2014), or by favouring organisms that prefer less acidic environments. For example, bacterial-feeding protozoans that were not negatively affected by Pb may benefit from increased food resources, since bacteria are known to prefer soils with high pH (Ingham et al. 1989, Alphei et al. 1996).

To summarise, soil faunal communities were affected by shooting-derived Pb at active and abandoned shooting ranges. In boreal forest soils, enchytraeid worms are the most sensitive group of the soil mesofauna. The effects of Pb are not always negative, since soil contaminants can also affect soil food webs indirectly, due to numerous linkages among biota and the abiotic environment. In soil food webs with numerous interactions, not only the soil fauna, but also soil microbes play a crucial role.

Microbial community (III)

As with the soil fauna, microbial communities were also affected by Pb, indicated by the microbial PLFA profile (Fig. 5). The clearest negative effect of Pb was detected in the soil fungi (Fig. 5, figures 3 & 4 in III), in accordance with studies concerning metal-contaminated soils (Pennanen et al. 1996, Kelly et al. 2003, Bååth et al. 2005, Hinojosa et al. 2005, Hui et al. 2012). However, due to the general view that fungi are more tolerant to metals than bacteria (Frostegård et al. 1993, Shi et al. 2002, Åkerblom et al. 2007), the negative impacts of metals on soil fungi have been suggested to arise from a decrease in mycorrhizal fungi due to metal-induced damages to roots and vegetation cover (Pennanen et al. 1996, Kelly et al. 2003, Bååth et al. 2005). It is also possible that in heterogeneously contaminated soils, fungi are exposed to the contaminant more so than bacteria because of their extensive mycelia in the soil – the exposure of one branch of fungal hyphae may affect a large part of the whole fungal organism (see Hinojosa et al. 2005).

The decreased fungal PLFA may also result from an increase in soil pH (see section 4.2 above), since soil fungi generally prefer lower pH than bacteria (Ingham et al. 1989). Soil pH is known to affect microbial community composition strongly, sometimes even stronger than heavy metals (Marcin et al. 2013).

In addition to changes in the microbial community, a consistent Pb-related increase in the ratios of cyclopropyl fatty acids and their precursors (cy17:0/16:1ω7c and cy19:0/18:1ω7c) was found. These ratios, along with the fungal/bacterial ratio, responded similarly to soil Pb contamination regardless of the sampling period, unlike absolute concentrations of various PLFA markers. This finding supports the notion of applying these ratios as biomarkers for environmental stress, not only in terms of nutrient deficiency (Zhang et al. 2006), or decreased pH (Zhang et al. 2006, Aliasgharzad et al. 2010), but also in terms of long-term metal exposure.

4.4 Effects of lead on soil processes

Are lead-induced changes in the soil food web reflected in soil processes (IV)?

Regardless of the clear effects of Pb on the soil food web (II, III), soil processes driven by the decomposer food web were only slightly affected. This indicates high resistance of boreal forest soils to this type of stress. In addition, soil processes were only weakly related to the soil food web structure, especially so to soil faunal abundances (Fig. 6). This was unexpected given the crucial role of the soil fauna in decomposition process and nutrient mineralisation (Setälä & Huhta 1991, Heneghan & Bolger 1998, Laakso & Setälä 1999,

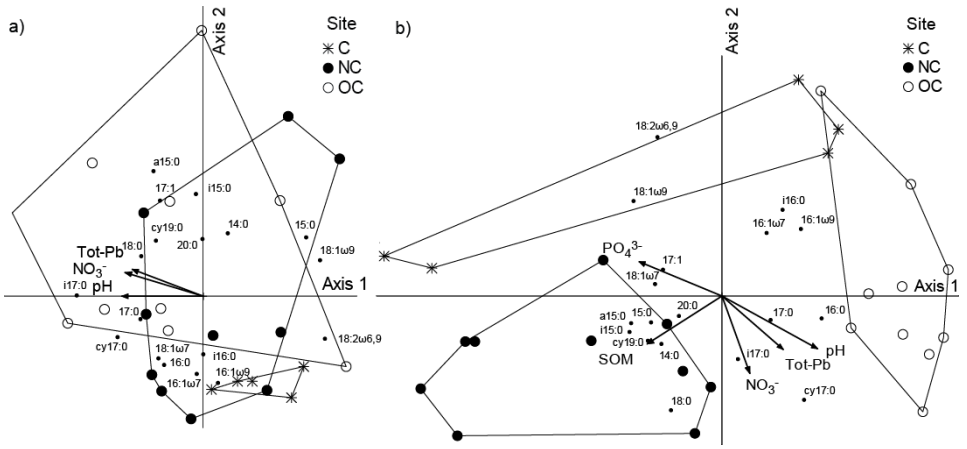

Figure 5. Redundancy analysis (RDA) of the PLFA data collected in H layer of the control site (C) and the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges in Autumn (a) and Spring II (b). Markers representing the study plots of one study site are enveloped with lines. PLFAs 18:2ω6,9 and 18:1ω9 are fungal biomarkers, while the sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, cy17:0, 17:00, 18:1ω7 and cy19:0 indicate bacteria (Paper III).

Setälä 2005). It is possible that due to the high degree of omnivory and functional redundancy in soil food webs (Laakso & Setälä 1999, Setälä 2005, Rohr et al. 2006), fauna that are sensitive to Pb are not directly linked to the functional attributes.

However, some relations between the soil food web structure and soil processes were detected (Fig. 6). Enchytraeid worm abundance had a slight positive relation to OC-grass decomposition and SIR, but only in the H layer, where the effects of Pb were so strong that enchytraeid worms were totally absent at OC with the highest Pb concentrations (II). In addition, the abundance of nematodes, as well as fungi and bacteria in the H-layer were associated with pine needle and NC-litter decomposition. This likely indicates the linkage between microbes and microbial-feeding nematodes in the food web and the crucial role of primary decomposers in decomposition processes.

In all, boreal forest soils seem to be relatively resistant to the stress induced by shooting-derived Pb, indicating functional redundancy among the soil biota in Pb-contaminated soils. Despite the negative effects of Pb on various soil fauna (II), there seems to be a sufficient number of biological interactions to maintain microbial populations active. However, if the abundance of enchytraeid worms decreases substantially, negative implications in soil processes can occur. Primary decomposers are of particular importance in soil processes. Therefore, changes in the microbial community due to direct toxic effects of Pb and indirect effects via increased pH, which favours bacteria over fungi, can be reflected in soil processes, as discussed below.

Microbial activity and potentially active microbial biomass (SIR) (IV)

Even though the microbial PLFA profile in the H layer differed between the study sites (III), in the entire organic soil layer (F + H) no statistically significant differences were found in microbial activity or potentially active microbial biomass, estimated by SIR (figure 2 in IV). However, basal respiration in Autumn was slightly higher at Control than at NC and both basal respiration and SIR responded negatively to soil Pb concentrations in the soil at some sampling events. Overall, the responses of microbial activity and SIR to metals seem to vary between studies (Bringmark et al. 1998, Niklinska et al. 1998, Salminen et al. 2002, Dai et al. 2004, Lazzaro et al. 2006).

The lack of strong responses in microbial activity to soil contamination suggests functional redundancy among microbes (Setälä & McLean 2004, Bardgett & Wardle 2010), since community composition in the current study changed due to Pb contamination (III). However, even the slight decrease in microbial activity detected in the present study may affect the degradation rate of organic matter in our study system.

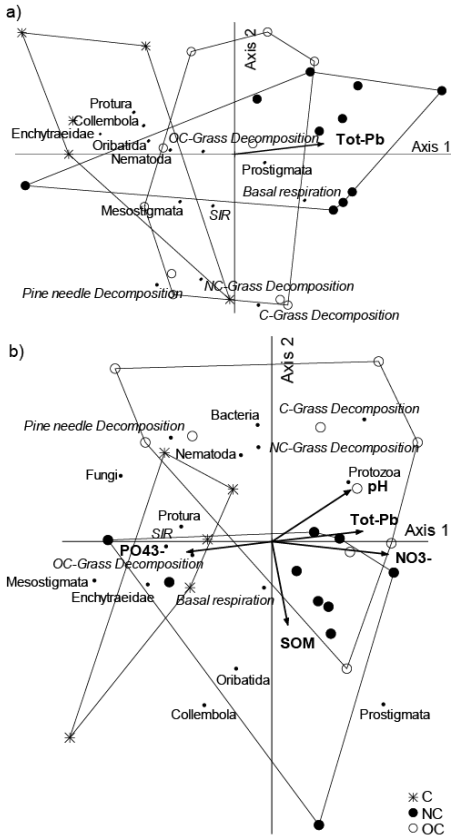

Figure 6. Redundancy analysis (RDA) with the structural (regular font) and functional (italics) variables as response variables, and soil properties (tot-Pb, pH, moisture, soil organic matter [SOM], NO_3^-, NH_4^+ and PO_4^{3-}) as potential explanatory variables (bold), analysed from soil samples taken from plots at the control site (C) and the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges in Spring II. Basal respiration and SIR were measured from the entire organic soil layer (F + H) (a, b), litter decomposition from the fermentation (F) layer (a, b), soil fauna from the F layer (a) and humus (H) layer (b) separately, and microbial biomasses only from the H layer (b) (Selonen & Setälä 2015).

Litter decomposition (IV)

Although the effects of Pb on litter decomposition were not strong, some signs of a decreased rate of decomposition were found. Pine needle litter decayed at a slower rate with increasing Pb concentrations and slight reductions were also found in the decomposition rate of grass litter with elevated concentrations of Pb (figures 2 & 4 in IV).

Pine needles contain an array of recalcitrant compounds such as lignin, the degradation of which is sensitive to metals and retard the decomposition process especially in the later stages of decomposition (Berg et al. 1991, McEnroe & Helmisaari 2001, Tuomela et al. 2005). Lignin is degraded by litter-decomposing fungi inhabiting the uppermost organic soil layers (Tuomela et al. 2005), whilst in grass litter, bacteria have a dominant role (Bardgett & Wardle 2010). Thus, the stronger negative effect of Pb on soil fungi than on bacteria (III) may explain why litter decomposition was retarded in pine needles but not in uncontaminated grasses. The closer location of the soil fungal PLFA to pine needle litter than to grass litter decomposition in the RDA ordination supports this conclusion (Fig. 6).

The negative effect of Pb on the decomposition of Pb-contaminated grass litter is in line with other studies in which the degradation of litter with elevated metal concentrations has been assessed (Berg et al. 1991, Post & Beeby 1996, Hui et al. 2009, but see Johnson & Hale 2004, Scheid et al. 2009). It seems that (i) Pb turned this otherwise easily decomposable grass material into a less

preferable resource for the decomposers and (ii) that the ability of this biota to decompose litter is impaired by Pb in the surrounding soil. Thus, decomposition rates of grass litter formed at these contaminated sites can be expected to be impaired, due to the clear positive correlation between Pb concentrations in the soil and in the leaves of the grass *C. arundinacea* (I).

A decreased rate of litter decomposition due to Pb-contamination can further lead to the accumulation of soil organic matter (SOM) (Sauve 2006) and impair nutrient mineralization in the soil (Post & Beeby 1996, Salminen et al. 2002, Dai et al. 2004). Lead-induced changes in soil nutrients and their leaching were also detected in the present study. However, many of these changes were related to other Pb-induced alterations, rather than decreased litter degradation rates, as discussed below.

Soil nutrients and nutrient leaching (III)

Shooting-derived Pb affected soil nutrients by increasing soil nitrate (NO_3^-) (Fig. 7), and decreasing soil phosphate (PO_4^{3-}) concentrations (Fig. 8). Lower PO_4^{3-} concentrations in the water extracts of Pb contaminated soils probably relates to the poor solubility of lead phosphates (Park et al. 2011), but may also indicate impaired nutrient mineralisation. The increased soil NO_3^-, in turn, suggests a decreased immobilisation to microbial and plant biomass, or increased nitrification rate, the transformation of ammonium (NH_4^+) into nitrite (NO_2^-) and further to nitrate (NO_3^-) by soil microbes. However, Pb has generally been found to inhibit nitrification (Rusk et al. 2004). Thus, the increased soil nitrate may be due to a higher soil pH. Nitrification rate in acidic pine forest soils is known to be positively related with soil pH (Nugroho et al. 2007), and the positive effect of pH on nitrification can be stronger than the negative effect of Pb (Sauve et al. 1999). Thus, the higher rate of nitrification due to an increased pH in the present study is a likely reason for the increased nitrate concentrations in these Pb-contaminated soils. However, decreased immobilisation by soil microbes or uptake by plants may also play a role, since the correlation between soil nitrate and pH was less significant as it was with soil Pb (figure 2 in III).

The increased nitrate concentrations detected at both contaminated sites were manifested as increased nitrate leaching only at OC (figure 6 in III). Similarly, ammonium (NH_4^+) leaching tended to be higher than the control only at OC (figure 6 in III). These findings suggest that, in the long-term, a crucial ecosystem service provided by soils, i.e. the ability of the system to retain nitrogen (Liiri et al. 2002b, Gordon et al. 2008), is gradually impaired due to Pb contamination.

Overall, in terms of nutrient dynamics, results of this thesis suggest that shooting-derived Pb can influence soil nutrients directly, by decreasing phosphate solubility and indirectly, by both increasing soil pH via oxidation of solid Pb and by inducing toxic effects on microbes and plants. Furthermore, since most fungi are efficient in immobilising nutrients (Bardgett et al., 2005, Gordon et al., 2008), the decreased fungal biomass due to toxic effects of Pb and increased soil pH can reduce nutrient

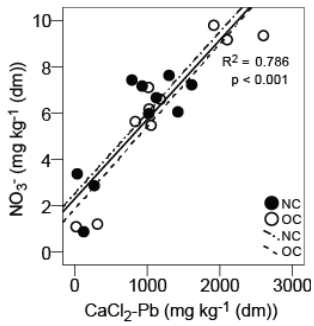

Figure 7. Relationship between NO_3^- and 0.01 M $CaCl_2$ extractable Pb in the H layer of the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges in Spring II (Paper III).

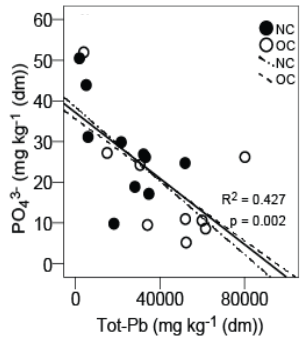

Figure 8. Relationship between PO_4^{3-} and total Pb in the H layer of the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges in Spring II (Paper III).

immobilisation by the fungi. Moreover, nutrient uptake by plants may also be reduced, if mycorrhizal fungi are violated. Thus, the adverse effects of Pb and increased pH on soil fungi and the positive effect of a higher soil pH on nitrification can generate the same outcome – increased soil nitrate concentrations and the leaching of nitrate from the organic soil layers.

4.5 Tree growth, needle nutrients and litter production (IV)

Even though soil nutrients responded similarly to soil Pb at the two contaminated sites, the pattern of radial growth of trees (*P. sylvestris*) diverged clearly between NC and OC (Fig. 9). Growth patterns of the trees reflected shooting history surprisingly well. At NC, the growth of pines was suppressed after shooting activity started in the late 1980s, while at the same time pine growth increased at OC when shooting ceased (Fig. 9). The decreased radial growth of pines at NC is in line with other studies at metal-contaminated sites (Mälkönen et al. 1999, Aznar et al. 2007, Fedorkov 2007, Aznar et al. 2009) and may derive from the toxic effects of Pb on tree roots and root-associating mycorrhizal fungi (see Mälkönen et al. 1999, Hartley-Whitaker et al. 2000, Kukkola et al. 2000, Menon et al. 2007, Sousa et al. 2014). In addition, Pb-induced changes in the soil food web (II, III) and in decomposition processes (IV) may contribute to decreased tree growth (see Mälkönen et al. 1999), bearing in mind that the lowest litter degradation rates were generally detected at NC (IV).

It is possible that the greater radial growth of pines at OC than at NC results from higher metal tolerance of the trees (see Stjernquist et al. 1998) and the somewhat lower tree stand density at the most contaminated location at OC (29 % lower than at Control and 56 % lower than at NC). Since the soil at OC was already contaminated when the forest stand started to regenerate naturally, Pb in the soil may have impeded seed germination and seedling survival (Kabir et al. 2008, Chudzinska et al. 2014). This

could have caused not only the lower stand density, but also higher metal tolerance of the surviving trees due to selection pressures towards metal tolerant individuals (Stjernquist et al. 1998, Chudzinska et al. 2014). At the active shooting range, such selection pressure at the time of stand generation did not occur, which may contribute to a higher susceptibility of tree growth to Pb when the shooting range opened.

Despite the greater radial growth at OC, nitrogen (N) content in pine needles was lower here than at NC and Control and phosphorus (P) content was below the deficiency level of 1.2 g kg^{-1} set for boreal forests (Mälkönen et al. 1999). Phosphorus deficiency may arise from the decreased phosphate availability observed in Pb-contaminated soils (III) due to, e.g. the low solubility of lead phosphates (Park et al. 2011). However, since also nitrogen content decreased in the needles, it is more likely that impaired nutrient uptake by the pines is the reason for low N and P concentrations in the needles. Similar observations have been reported in other studies conducted in metal-contaminated soils (Arduini et al. 1998, Mälkönen et al. 1999, Jentschke & Goldbold 2000, Kim et al. 2003, Dominguez et al. 2010, Parraga-Aguado et al. 2014). It is possible that trees were unable to take up soil nutrients due to the toxic effects of heavy metals on tree roots and the mycorrhizal fungi (Mälkönen et al. 1999, Hartley-Whitaker et al. 2000, Kukkola et al. 2000, Menon et al. 2007, Sousa et al. 2014). Increased nitrogen leaching at OC (III) also suggests that trees were unable to exploit nutrients efficiently at the site.

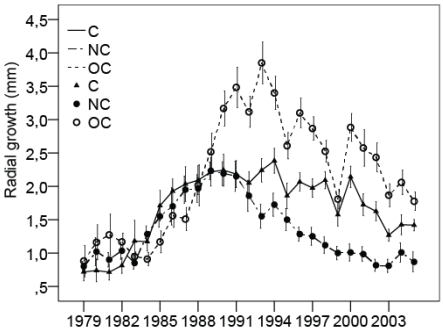

Figure 9. Radial growth (mean ± SE) of Scots pine (*Pinus sylvestris*) at the control site (C) and the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges, measured as the width of annual tree rings (n = 12) (Selonen & Setälä 2015).

Nutrient deficiency in Scots pine may further contribute to the observed increased litter production at OC due to a decrease in longevity of older needles (see Mälkönen et al. 1999). Increased litter production may also result directly from Pb exposure, since needle damage and loss have been reported at metal-contaminated sites (Stjernquist et al. 1998, Fedorkov et al. 2007).

These findings imply that Pb-induced changes in the tree stand can be surprisingly divergent between an abandoned shooting range and an active one. Differences between the two Pb-contaminated sites emphasise the complex nature of interactions within the soil food web and between the soil and aboveground systems. In essence, divergent contamination histories between the two sites can bring about differences not only in seed germination and seedling survival during the regeneration of a forest, but also contributes to the vertical distribution and availability of Pb in soils (I) at the latter phases of stand development, as discussed in the following section.

4.6 Towards ecosystem recovery?

Despite the presence of a barrier (backstop) between the shooting location at the active shooting range and the adjacent pine forest, substantial amounts of pellets were still falling behind the backstop at NC (figure 2 in I). Consequently, the continuous input of Pb pellets on the soil surface maintains the high level of Pb in the topmost layer of the soil. However, even though an equal amount of Pb pellets was found in the organic soil horizon at both contaminated sites, tot-Pb concentrations in the F layer were substantially lower at OC than at NC (I).

Lower concentrations of Pb in the F layer at OC most probably derive from the formation of new soil material due to litter accumulation on the soil surface. In addition, it is likely that Pb pellets have migrated deeper into the F layer due to gravity. Since the decomposition of organic matter in mor-type organic soils is slow and the intensity of soil mixing is low due to a lack of earthworms that are able to transport contaminants to the soil surface from deeper soil layers (Haimi 2000, Eijsackers et al. 2008), a less contaminated surface soil layer is gradually formed. The present study provides evidence that such a surface soil layer is utilized by the soil fauna (II) and can promote recovery of soil processes (IV). Various signs of recovery were found from tissue Pb concentrations (I) faunal abundances (II) and litter decomposition rates (IV).

The accumulation of Pb in some of the studied biota had decreased after range abandonment. For example, Pb concentrations in blueberry leaves and enchytraeid worms at OC were

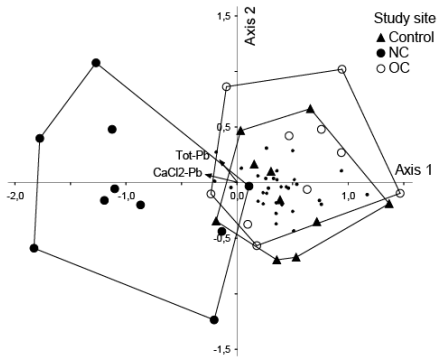

Figure 10. NMDS ordination of the microarthropod community at the active (new contaminated [NC]) and abandoned (old contaminated [OC]) shooting ranges, and at the control site in Spring I. Polygons envelope observations of a particular study site. Each small dot represents one taxon. (Selonen et al. 2014).

approximately half and in pine needles approximately one third of those at NC (table 3 in I, figure 5 in I). Among the soil fauna the clearest signs of recovery were found in microarthropods. When the F and H layers were combined, the abundances of microarthropod taxa at OC were generally higher than at NC (Online Resource 2 in II). In addition, at NC some microarthropod groups existed relatively deeper in the soil. Furthermore, microarthropod community composition at OC was similar to that at the Control especially in Spring I (Fig. 10), which indicates that recovery of biota to a pre-disturbed state is possible even when the disturbance is not entirely ceased (see Matthews et al. 1996, Clements & Newman 2002, Van Straalen 2003, Clements & Rohr 2009).

It is well known that a number of soil fauna can avoid metal contamination (da Luz et al. 2004, Lukkari & Haimi 2005, Amorim et al. 2008), and that plants growing in metal-contaminated

soils can utilize less contaminated microhabitats (Turner & Dickinson 1993, Ginocchio et al. 2004). However, in the present study the role of avoidance cannot be separated from the role of toxic effects of Pb. Whatever the case, the various differences between the active and abandoned shooting range depend on Pb concentration in soil. This is indicated by the almost identical responses of Pb concentrations in plant leaves, the abundances of some soil faunal groups and fungal biomass to soil Pb concentration at the two contaminated sites (figure 4 in II, figure 4 in III). Thus, the decreased concentration of Pb in the topmost organic soil layer is likely responsible for the signs of recovery at the abandoned shooting range.

The recovery of soil communities was also reflected in the rates of pine needle litter decomposition (IV). Saprotrophic fungi that play a crucial role in needle litter decomposition inhabit, largely, the uppermost soil profile (Lindahl et al. 2007). Thus, the negative effects of Pb on litter-decomposing fungi are likely to be stronger at the active shooting range, where a substantial number of Pb pellets occurs on the soil surface. Although fungal biomass was only measured in the H layer, it is probable that the decreased Pb concentrations in the F layer at OC promoted fungal growth, since the soil fungal PLFA correlated negatively with soil Pb at both contaminated sites (III).

To summarise, decreased Pb concentrations in the uppermost soil layer and in some of the biota sampled, as well as increased abundances of some of the soil fauna and an increase in the decomposition rate of pine needle litter suggest recovery in the topmost soil layer at the abandoned shooting range. This indicates that boreal forest soils are relatively resilient to this type of disturbance and that recovery is possible, at least to some extent (see Matthews et al. 1996, Clements & Newman 2002, Van Straalen 2003, Clements & Rohr 2009). However, since the corrosion of Pb pellets continues deeper in the soil profile, recovery seems to be restricted to the upper parts of the organic soil layer. Moreover, Pb has not completely disappeared even at the uppermost L and F layers, but continues to be cycled in the forest ecosystem between the soil, biota and the organic residues of these organisms, as discussed below.

4.7 Gradual release of lead

Release of lead via the food web

Contrary to expectations, concentrations of H_2O-Pb did not differ in the upper organic layer between the two contaminated sites (I). In fact, H_2O-Pb at OC tended to be higher in the uppermost soil horizon than in the H layer, although substantially more tot-Pb was found in the H layer (I). This indicates that H_2O extractable Pb in the uppermost organic soil horizon does not solely and directly derive from weathering pellets, but also from the decomposing litter once entered in the food web. This hypothesis is supported by findings that metal cations can be translocated from deeper soil horizons into surface soils by plants and fungi, taken up first by roots or fungal hyphae and then released via litter fall and hyphal death (Klaminder et al. 2005, Mertens et al. 2007, Clarholm &

Skyllberg 2013, Parraga-Aguado et al. 2014). Furthermore, metal concentrations in the decaying litter tend to increase with time, when metals enrich in the decomposing material (Berg et al. 1991, Lomander & Johansson 2001, Johnson & Hale 2004, Scheid et al. 2009).

Cao et al. (2003b) suggested that Pb released from decomposing plant material at shooting range sites is highly bioavailable, and due to litter accumulation, the bioavailability of Pb may gradually increase in the soil. However, given the clear signs of soil food web recovery in the topmost soil layer in the present study, this pathway of Pb seems to be insignificant. Furthermore, concentrations of Pb in plant leaves and soil faunal abundances generally correlated stronger with tot-Pb than H_2O-Pb (I, II, III). These findings suggest that bioavailability of Pb in the surface soil decreases with time, even though H_2O-Pb seems to remain at the same level. However, based on results presented here, it cannot be ruled out that the recovery of a Pb-contaminated soil system may, to some extent, depend on the cycling of Pb between the soil, plants and the soil biota.

Release of lead via corroding pellets

Even though the biota and processes in the topmost soil layer seem to gradually recover after range abandonment, tot-Pb concentrations and soil toxicity deeper in the H layer increase with time. Both the soil faunal (II) and microbial (III) communities in the H layer at the control site differed more from those at OC than at NC. Decreased abundances in the H layer of OC were found in the soil fungi (III), oribatid mites, springtails and the total amount of microarthropods (II). Moreover, enchytraeid worms, a key taxon in these boreal pine forests, were totally absent from the H layer at the abandoned shooting range (II). As discussed above, decreased abundances of the soil fauna can be due to the toxic effects of Pb, avoidance behaviour (Lukkari & Haimi 2005, Amorim et al. 2008, Owojori et al. 2011), or both. In any event, the decreased faunal abundances and fungal biomass in the H layer can be reflected in soil processes. Soil processes carried out by euedaphic species can be impaired, since the distribution – and thus the functional importance – of these species is restricted in the deeper layers of the organic soil horizon (Berg 2010).

The increasing negative effects of Pb on soil biota in the H layer after range abandonment may further have affected nutrient uptake by Scots pine (*P. sylvestris*). The decreased nitrogen and phosphorus content in pine needles at OC (IV) may arise from the increased toxic effects of Pb on ectomycorrhizal fungi (Hartley-Whitaker et al. 2000, Sousa et al. 2014), since mycorrhizal fungi dominate deeper organic soil layers in boreal pine forest soils (Lindahl et al. 2007). The impairment of nitrogen uptake may also contribute to increased nitrogen leaching from the ecosystem at OC (III). This indicates that a crucial ecosystem service provided by the soil – the ability to retain nutrients – is impaired at abandoned shooting ranges with time.

Furthermore, not only nitrogen leaching, but also leaching of Pb through the organic soil horizon increases with time – leaching of Pb at OC was twice as

high as at NC (figure 6 in I). This increased Pb leaching apparently results from the continuous release of Pb from corroding pellets in the soil (see Levonmäki et al. 2006, Takamatsu et al. 2010) and the gradual movement of Pb downwards (Hartikainen & Kerko 2009). Bearing in mind that one-third of all shooting ranges in Finland are situated close to aquifers supporting groundwater reservoirs (Sorvari et al. 2006), the continued leaching of Pb at old shooting range sites is likely to increase the risks to ground water quality.

5. CONCLUSIONS

This thesis provides a broad overview on the fate and impacts of a contaminant at the ecosystem level, and thus incorporates more "eco" into ecotoxicological research. Furthermore, the close proximity of the active (NC) and abandoned (OC) shooting ranges and the uncontaminated control site in the same pine forest stand enabled to assess whether the fate and effects of Pb change with time after the contaminating activity has ceased. Due to this approach, the ecological concepts of ecosystem resistance and resilience could be observed from an ecotoxicological perspective. The major conclusions of this research are presented below.

Firstly, a substantial amount of lead pellets can accumulate in ecosystems nearby shooting range sites regardless of "preventative" backstops. Although the relative availability of lead (Pb) to the biota is low even decades after shooting activities have ceased, Pb can nevertheless bioaccumulate and cycle between the soil, plants and the soil biota.

Secondly, Pb affects soil food webs directly due to toxic effects, but also indirectly due to the numerous interactions within and between biota and the abiotic environment. The most sensitive group of soil fauna was enchytraeid worms that represent a keystone species in the process of decomposition in boreal forest soils. Not only negative, but also positive responses to soil Pb were found among the soil fauna, indicating indirect effects of Pb through changes, e.g. in food resources, competition or predation pressure. In the microbial community, soil fungi were generally more sensitive to Pb than bacteria. A decrease in the soil fungal biomass may result both from the direct toxic effects of Pb, but also from an increase in soil pH, which favours bacteria over fungi.

Thirdly, despite of clear Pb-induced changes in the soil food web, only slight changes were detected in soil processes. This suggests that functional redundancy is common among the soil biota and indicates high resistance of the boreal forest ecosystem to disturbances caused by shooting-derived Pb. However, pine needle litter decomposition was retarded by Pb, likely due to direct and indirect negative impacts of Pb on soil saprotrophic fungi. In addition, soil nutrient concentrations were clearly affected by Pb. Some of these changes, like reduced phosphate solubility derives directly from increased Pb concentrations in the soil. However, some of the changes in soil nutrients likely result from indirect effects of Pb. The toxic effect of Pb on plants and soil fungi may have decreased nitrate uptake

and immobilisation. In addition, Pb-induced increase in soil pH likely has increased nitrification rate and affected nutrient immobilisation. Impaired nutrient dynamics are also shown in the nutrient deficiency of Scots pines and possibly also as decreased growth of pine trees. However, tree growth was retarded only at the active shooting range but stimulated at the abandoned site, indicating that the effects of Pb on the structure and functions of boreal forest ecosystems are strongly dependent on the contamination history of the site.

Fourthly, the fate and effects of shooting-derived Pb in boreal ecosystems change in the long-term after range abandonment. One major factor determining these changes is the gradually changing vertical distribution of Pb in the biologically active organic soil layer due to slow decomposition rates and a lack of soil mixing in mor-type boreal forest soils. At abandoned shooting ranges, litter accumulation on the soil surface over time enables the formation of less contaminated topmost soil layer, while the gradual release of Pb from pellets that have subsided in the soil increases Pb concentrations deeper in the organic soil horizon. The less contaminated uppermost soil layer can evidently provide habitat to various soil organisms, and signs of recovery were observed in faunal communities as well as in the decomposition process. This suggests that boreal forest soils are relatively resilient to this type of disturbance and that recovery in topmost soil layer is possible. On the other hand, toxicity of the humus layer deeper in the soil increases with time – the absence of enchytraeid worms in the humus layer of the abandoned shooting range being the most striking evidence of this. Many of the measured variables correlated surprisingly well with soil Pb concentrations, often similarly at the two contaminated sites. Thus, differentiation of the vertical distribution of Pb in soils over time explains the various signs of recovery in the fermentation layer, but also the increased soil toxicity of the humus layer. In addition, as leaching of Pb appeared to increase with time, the risks to ground water quality are likely to increase with time as well.

ACKNOWLEDGEMENTS

I thank my supervisors Heikki Setälä and Rauni Strömmer for their advice, patience and contribution to this work, as well as Mira Liiri for her assistance and support as a co-author, as a mentor and as a friend. I am grateful for Jari Haimi for introducing me to the world of soil ecotoxicology. I am thankful also for many advices got during my work, especially from Olli-Pekka Penttinen, Jukka Pellinen, Gaia Francini and Marja Mattsson. I also thank Lahden ampumaseura (LAS) for co-operation and Johan Kotze for checking the language.

I am thankful to all the people that participated in the practical work. Jukka Pellinen, Santeri Savolainen, Johanna Kilpi-Koski, Anu Liukkonen, Ari Väisänen, Tiina Kontkanen and Janne Silvonen contributed in the Pb analytics. Mia Suontaus performed the PLFA analyses and Marianne Lehtonen the nutrient analyses. Tuukka Ryynänen, Sandro Heide, Ville Selonen, Hui Nan and Aki Sinkkonen contributed in the field work along with the co-authors.

Hui Nan also drew the initial schematic sketch of the area, according to which Figure 1 was drawn. Many pairs of hands were also helping me in the laborious work of picking the pellets from the soil samples.

This study was funded by Helsinki University Research Centre (HERC, currently HENVI); Maj and Tor Nessling Foundation; Finnish Cultural Foundation, Päijät-Häme Regional fund; Onni ja Hilja Tuovisen Säätiö; Kone Foundation; and Finnish Cultural Foundation.

Lastly, I would like to thank for all the support I have got from my friends and family. Thank you, my friends at work – especially Marleena and XinXin – for cheering me up and giving me support. Thank you, my friends got when living in Kalajoki, Jyväskylä and Lahti. Thank you, my mother and father, dear sisters Jonna and Hanna and my parents in law for all the support. And last, but not least – Thank you, my dear, dear family – Matias, Viljami, Inkeri and Ville.

REFERENCES

Abrahamsen, G. 1973: Studies on Body-Volume, Body-Surface Area, Density and Live Weight of Enchytraeidae (Oligochaeta). – *Pedobiologia* 13: 6-15.

Åkerblom, S., Bååth, E., Bringmark, L. & Bringmark, E. 2007: Experimentally induced effects of heavy metal on microbial activity and community structure of forest mor layers. – *Biol. Fert. Soils* 44: 79-91.

Aliasgharzad, N., Martensson, L. & Olsson, P. A. 2010: Acidification of a sandy grassland favours bacteria and disfavours fungal saprotrophs as estimated by fatty acid profiling. – *Soil Biol. Biochem.* 42: 1058-1064.

Alphei, J., Bonkowski, M. & Scheu, S. 1996: Protozoa, Nematoda and Lumbricidae in the rhizosphere of Hordelymus europaeus (Poaceae): Faunal interactions, response of microorganisms and effects on plant growth. – *Oecologia* 106: 111-126.

Amorim, M. J. B., Novais, S., Römbke, J. & Soares, A. M. V. M. 2008: Enchytraeus albidus (Enchytraeidae): A test organism in a standardised avoidance test? Effects of different chemical substances. – *Environ. Int.* 34: 363-371.

Anderson, J. P. E. & Domsch, K. H. 1978: A physiological method for the quantitative measurement of microbial biomass in soils. – *Soil Biol. Biochem.* 10: 215-21.

Anderson, M. 2001: A new method for non-parametric multivariate analysis of variance. – *Austral Ecol.* 26: 32-46.

Arduini, I., Godbold, D., Onnis, A. & Stefani, A. 1998: Heavy metals influence mineral nutrition of tree seedlings. – *Chemosphere* 36: 739-744.

Aznar, J. -., Richer-Lafleche, M., Begin, C. & Marion, J. 2007: Mining and smelting activities produce anomalies in tree-growth patterns (Murdochville, Quebec). – *Water Air Soil Pollut.* 186: 139-147.

Aznar, J. -., Richer-Lafleche, M., Paucar-Munoz, H., Bordeleau, M. & Begin, Y. 2009: Is tree growth reduction related to direct foliar injuries or soil chemistry modifications?. – *Chemosphere* 76: 1366-1371.

Bååth, E., Diaz-Ravina, M. & Bakken, L. 2005: Microbial biomass, community structure and metal tolerance of a naturally Pb-enriched forest soil. – *Microb. Ecol.* 50: 496-505.

Bååth, E., Frostegård, Å., Diaz-Ravina, M. & Tunlid, A. 1998: Microbial community-based measurements to estimate heavy metal effects in soil: The use of phospholipid fatty acid patterns and bacterial community tolerance. – *Ambio* 27: 58-61.

Bardgett, R., Bowman, W., Kaufmann, R. & Schmidt, S. 2005: A temporal approach to linking aboveground and belowground

ecology. – *Trends Ecol. Evol.* 20: 634-641.
Bardgett, R. D. & Wardle, D. A. 2010: *Aboveground-belowground linkages: Biotic Interactions, ecosystem processes, and global change*. Oxford University Press, USA.
Begon, M., Townsend, C. R. & Harper, J. L. 2005: *Ecology: From Individuals to Ecosystems*. Wiley-Blackwell.
Berard, A., Mazzia, C., Sappin-Didier, V., Capowiez, L. & Capowiez, Y. 2014: Use of the MicroResp (TM) method to assess Pollution-Induced Community Tolerance in the context of metal soil contamination. – *Ecol. Ind.* 40: 27-33.
Berg, M. 2010: Spatio-temporal structure in soil communities and ecosystem processes. In: Verhoef, H. and Morin, P. (eds.), *Community Ecology, Processes, Models, and Applications*: 69-79. Oxford University Press. USA.
Berg, B., Ekbohm, G., Söderström, B. & Staaf, H. 1991: Reduction of Decomposition Rates of Scots Pine Needle Litter due to Heavy-Metal Pollution. – *Water Air Soil Pollut.* 59: 165-177.
Binkley, D. & Fisher, R. F. 2013: *Ecology and Management of Forest Soils*. John Wiley & Sons. Singapore.
Bligh, E. & Dyer, W. 1959: A rapid method of total lipid extraction and purification. – *Can. J. Biochem. Phys.* 37: 911-917.
Bongers, T., Ilieva-Makulec, K. & Ekschmitt, K. 2001: Acute sensitivity of nematode taxa to $CuSO_4$ and relationships with feeding-type and life-history classification. – *Environ. Toxicol. Chem.* 20: 1511-1516.
Bossio, D. & Scow, K. 1998: Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. – *Microb. Ecol.* 35: 265-278.
Bradham, K., Dayton, E., Basta, N., Schroder, J., Payton, M. & Lanno, R. 2006: Effect of soil properties on lead bioavailability and toxicity to earthworms. – *Environ. Toxicol. Chem.* 25: 769-775.
Breure, A. M., Groot, M. & Eijsackers, H. J. P. 2008: System-oriented ecotoxicological research: Which way to go? – *Sci. Total Environ.* 406: 530-536.
Bringmark, L., Bringmark, E. & Samuelsson, B. 1998: Effects on mor layer respiration by small experimental additions of mercury and lead. – *Sci. Total Environ.* 213: 115-119.
Briones, A. & Reichardt, W. 1999: Estimating microbial population counts by 'most probable number' using Microsoft Excel (R). – *J. Microbiol. Methods* 35: 157-161.
Cao, X., Ma, L., Chen, M., Hardison, D. & Harris, W. 2003a: Lead transformation and distribution in the soils of shooting ranges in Florida. – *Sci. Total Environ.* 307: 179-189.
Cao, X., Ma, L., Chen, M., Hardison, D. & Harris, W. 2003b: Weathering of lead bullets and their environmental effects at outdoor shooting ranges. – *J. Environ. Qual.* 32: 526-534.
Chudzinska, E., Diatta, J. B. & Wojnicka-Poltorak, A. 2014: Adaptation strategies and referencing trial of Scots and black pine populations subjected to heavy metal pollution. – *Environ. Sci. Pollut. Res.* 21: 2165-2177.
Clarholm, M. & Skyllberg, U. 2013: Translocation of metals by trees and fungi regulates pH, soil organic matter turnover and nitrogen availability in acidic forest soils. – *Soil Biol. Biochem.* 63: 142-153.
Clements, W. H. & Newman, M. C. 2002: *Community Ecotoxicology*. John Wiley & Sons. Chichester, West Sussex, England.
Clements, W. H. & Rohr, J. R. 2009: Community Responses to Contaminants: using Basic Ecological Principles to Predict Ecotoxicological Effects. – *Environ. Toxicol. Chem.* 28: 1789-1800.
Coleman, D. C., Crossley, J. D. A. & Hendrix, P. F. 2004: *Fundamentals of Soil Ecology*. Elsevier Academic Press. USA.
da Luz, T., Ribeiro, R. & Sousa, J. 2004: Avoidance tests with Collembola and earthworms as early screening tools for site-specific assessment of polluted soils. – *Environ. Toxicol. Chem.* 23: 2188-2193.
Dai, J., Becquer, T., Rouiller, J., Reversat, G., Bernhard-Reversat, F. & Lavelle, P. 2004: Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. – *Appl. Soil Ecol.* 25: 99-109.
Darling, C. & Thomas, V. 2003: The distribution of outdoor shooting ranges in Ontario and the potential for lead pollution of soil and water. – *Sci. Total Environ.* 313: 235-243.
DeAngelis, D. L. 2009: Boreal forest. In: Jorgensen, S. E. (ed.), *Ecosystem Ecology*:

181-183. Elsevier. Amsterdam, The Netherlands.
Didden, W. & Römbke, J. 2001: Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. – *Ecotoxicol. Environ. Saf.* 50: 25-43.
Dominguez, M. T., Maranon, T., Murillo, J. M., Schulin, R. & Robinson, B. H. 2010: Nutritional Status of Mediterranean Trees Growing in a Contaminated and Remediated Area. – *Water Air Soil Pollut.* 205: 305-321.
Dufrene, M. & Legendre, P. 1997: Species assemblages and indicator species: The need for a flexible asymmetrical approach. – *Ecol. Monogr.* 67: 345-366.
Ehrlich, P. & Walker, B. 1998: Rivets and redundancy. – *Bioscience* 48: 387-387.
Eijsackers, H. J. P., Groot, M. & Breure, A. M. 2008: Upgrading system-oriented ecotoxicological research. – *Sci. Total Environ.* 406: 373-384.
Ekelund, F. 1998: Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. – *Soil Biol. Biochem.* 30: 1343-1347.
Faber, J. H., Creamer, R. E., Mulder, C., Rombke, J., Rutgers, M., Sousa, J. P., Stone, D. & Griffiths, B. S. 2013: The practicalities and pitfalls of establishing a policy-relevant and cost-effective soil biological monitoring scheme. – *Integrated environmental assessment and management* 9: 276-84.
Fedorkov, A. 2007: Effect of heavy metal pollution of forest soil on radial growth of Scots pine. – *For. Pathol.* 37: 136-142.
Fedorkov, A., Kaitera, J. & Jalkanen, R. 2007: Condition and growth of Scots pine seedlings under strong and weak pollution in Kola Peninsula. – *Baltic For.* 13: 179-183.
Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A. & Cleveland, C. C. 2009: Global patterns in belowground communities. – *Ecol. Lett.* 12: 1238-1249.
Filser, J. 2002: The role of Collembola in carbon and nitrogen cycling in soil. – *Pedobiologia* 46: 234-245.
Filser, J., Koehler, H., Ruf, A., Römbke, J., Prinzing, A. & Schaefer, M. 2008: Ecological theory meets soil ecotoxicology: Challenge and chance. – *Basic Appl. Ecol.* 9: 346-355.
Filser, J. 2008: Ecotoxicology and ecosystems: Relevance, restrictions, research needs. – *Basic Appl. Ecol.* 9: 333-336.
Frostegård, Å. & Bååth, E. 1996: The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. – *Biol. Fert. Soils* 22: 59-65.
Frostegård, Å., Tunlid, A. & Bååth, E. 1993: Phospholipid fatty-acid composition, biomass, and activity of microbial communities from 2 soil types experimentally exposed to different heavy-metals. – *Appl. Environ. Microbiol.* 59: 3605-3617.
Frostegård, Å., Tunlid, A. & Bååth, E. 2011: Use and misuse of PLFA measurements in soils. – *Soil Biol. Biochem.* 43: 1621-1625.
Frouz, J., Prach, K., Pizl, V., Hanel, L., Stary, J., Tajovsky, K., Materna, J., Balik, V., Kalcik, J. & Rehounkova, K. 2008: Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. – *Eur. J. Soil Biol.* 44: 109-121.
Georgieva, S., McGrath, S., Hooper, D. & Chambers, B. 2002: Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. – *Appl. Soil Ecol.* 20: 27-42.
Giller, K. E., Witter, E. & McGrath, S. P. 2009: Heavy metals and soil microbes. – *Soil Biol. Biochem.* 41: 2031-2037.
Giller, P. S. 1996: The diversity of soil communities, the 'poor man's tropical rainforest'. – *Biodivers. Conserv.* 5: 135-168.
Ginocchio, R., Carvallo, G., Toro, I., Bustamante, E., Silva, Y. & Sepulveda, N. 2004: Micro-spatial variation of soil metal pollution and plant recruitment near a copper smelter in Central Chile. – *Environ. Pollut.* 127: 343-352.
Gordon, H., Haygarth, P. M. & Bardgett, R. D. 2008: Drying and rewetting effects on soil microbial community composition and nutrient leaching. – *Soil Biol. Biochem.* 40: 302-311.
Hågvar, S. & Abrahamsen, G. 1990: Microarthropoda and Enchytraeidae (Oligochaeta) in Naturally Lead-Contaminated Soil - a Gradient Study. – *Environ. Entomol.* 19: 1263-1277.
Haimi, J. 2000: Decomposer animals and bioremediation of soils. – *Environ. Pollut.* 107: 233-238.
Haimi, J. & Siira-Pietikäinen, A. 1996: Decomposer animal communities in forest

soil along heavy metal pollution gradient. – *Fresenius J. Anal. Chem.* 354: 672-675.
Haimi, J. & Mätäsniemi, L. 2002: Soil decomposer animal community in heavy-metal contaminated coniferous forest with and without liming. – *Eur. J. Soil Biol.* 38: 131-136.
Hartikainen, H. & Kerko, E. 2009: Lead in various chemical pools in soil depth profiles on two shooting ranges of different age. – *Boreal Environ. Res.* 14: 61-69.
Hartley-Whitaker, J., Cairney, J. & Meharg, A. 2000: Toxic effects of cadmium and zinc on ectomycorrhizal colonization of Scots pine (Pinus sylvestris L.) from soil inoculum. – *Environ. Toxicol. Chem.* 19: 694-699.
Heneghan, L. & Bolger, T. 1998: Soil microarthropod contribution to forest ecosystem processes: the importance of observational scale. – *Plant Soil* 205: 113-124.
Hinojosa, M., Carreira, J., Garcia-Ruiz, R. & Dick, R. 2005: Microbial response to heavy metal-polluted soils: Community analysis from phospholipid-linked fatty acids and ester-linked fatty acids extracts. – *J. Environ. Qual.* 34: 1789-1800.
Houba, V., Lexmond, T., Novozamsky, I. & vanderLee, J. 1996: State of the art and future developments in soil analysis for bioavailability assessment. – *Sci. Total Environ.* 178: 21-28.
Huhta, V., Persson, T. & Setälä, H. 1998: Functional implications of soil fauna diversity in boreal forests. – *Appl. Soil Ecol.* 10: 277-288.
Hui, C. A. 2002: Lead Distribution throughout Soil, Flora, and an Invertebrate at a; Wetland Skeet Range. – *J. Toxicol. Environ. Health Part A* 65: 1093-1107.
Hui, N., Liu, X., Kurola, J., Mikola, J. & Romantschuk, M. 2012: Lead (Pb) contamination alters richness and diversity of the fungal, but not the bacterial community in pine forest soil. – *Boreal Environ. Res.* 17: 46-58.
Hui, N., Jumpponen, A., Niskanen, T., Liimatainen, K., Jones, K. L., Koivula, T., Romantschuk, M. & Strömmer, R. 2011: EcM fungal community structure, but not diversity, altered in a Pb-contaminated shooting range in a boreal coniferous forest site in Southern Finland. – *FEMS Microbiol. Ecol.* 76: 121-132.
Hui, N., Selonen, S., Hanzel, J., Tuomela, M., Rainio, A., Kontio, H., Hakala, K., Lankinen, P., Steffen, K., Fingerroos, T., Strömmer, R., Setälä, H., Hatakka, A. & Romantschuk, M. 2009: Influence of lead on organisms within the detritus food web of a contaminated pine forest soil. – *Boreal Environ. Res.* 14: 70-85.
Ingham, E., Coleman, D. & Moore, J. 1989: An Analysis of Food-Web Structure and Function in a Sortgrass Prairie, a Mountain Meadow, and a Lodgepole Pine Forest. – *Biol. Fert. Soils* 8: 29-37.
Jentschke, G. & Goldbold, D. 2000: Metal toxicity and ectomycorrhizas. – *Physiol. Plantarum* 109: 107-116.
Johnson, D. & Hale, B. 2004: White birch (Betula papyrifera Marshall) foliar litter decomposition in relation to trace metal atmospheric inputs at metal-contaminated and uncontaminated sites near Sudbury, Ontario and Rouyn-Noranda, Quebec, Canada. – *Environ. Pollut.* 127: 65-72.
Kabir, M., Iqbal, M. Z., Shafiq, M. & Farooqi, Z. R. 2008: Reduction in Germination and Seedling Growth of Thespesia Populnea L., Caused by Lead and Cadmium Treatments. – *Pak. J. Bot.* 40: 2419-2426.
Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R. & Kaushik, R. 2005: Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. – *Curr. Sci.* 89: 1103-1112.
Kelly, J., Häggblom, M. & Tate, R. 2003: Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. – *Biol. Fert. Soils* 38: 65-71.
Khalil, M. A., Janssens, T. K. S., Berg, M. P. & van Straalen, N. M. 2009: Identification of metal-responsive oribatid mites in a comparative survey of polluted soils. – *Pedobiologia* 52: 207-221.
Kim, C., Bell, J. & Power, S. 2003: Effects of soil cadmium on Pinus sylvestris L. seedlings. – *Plant Soil* 257: 443-449.
Klaminder, J., Bindler, R., Emteryd, O. & Renberg, I. 2005: Uptake and recycling of lead by boreal forest plants: Quantitative estimates from a site in northern Sweden. – *Geochim. Cosmochim. Acta* 69: 2485-2496.

Kools, S. A. E., Boivin, M. Y., Van Der Wurff, A. W. G., Berg, M. P., Van Gestel, C. A. M. & Van Straalen, N. M. 2009: Assessment of structure and function in metal polluted grasslands using Terrestrial Model Ecosystems. – *Ecotoxicol. Environ. Saf.* 72: 51-59.

Korthals, G., Alexiev, A., Lexmond, T., Kammenga, J. & Bongers, T. 1996: Long-term effects of copper and pH on the nematode community in an agroecosystem. – *Environ. Toxicol. Chem.* 15: 979-985.

Kukkola, E., Rautio, P. & Huttunen, S. 2000: Stress indications in copper- and nickel-exposed Scots pine seedlings. – *Environ. Exp. Bot.* 43: 197-210.

Laakso, J. & Setälä, H. 1999: Sensitivity of primary production to changes in the architecture of belowground food webs. – *Oikos* 87: 57-64.

Labare, M. P., Butkus, M. A., Riegner, D., Schommer, N. & Atkinson, J. 2004: Evaluation of lead movement from the abiotic to biotic at a small-arms firing range. – *Environ. Geol. (Berlin)* 46: 750-754.

Lahr, J., Kools, S. A. E., van der Hout, A. & Faber, J. H. 2008: Combined effects of zinc and earthworm density on soil ecosystem functioning. – *Soil Biol. Biochem.* 40: 334-341.

Lazzaro, A., Schulin, R., Widmer, F. & Frey, B. 2006: Changes in lead availability affect bacterial community structure but not basal respiration in a microcosm study with forest soils. – *Sci. Total Environ.* 371: 110-124.

Legendre, P. & Legendre, L. 1998: *Numerical Ecology*. Elsevier. Amsterdam.

Levonmäki, M., Hartikainen, H. & Kairesalo, T. 2006: Effect of organic amendment and plant roots on the solubility and mobilization of lead in soils at a shooting range. – *J. Environ. Qual.* 35: 1026-1031.

Lewis, L. A., Poppenga, R. J., Davidson, W. R., Fischer, J. R. & Morgan, K. A. 2001: Lead Toxicosis and Trace Element Levels in Wild Birds and Mammals at a Firearms Training Facility. – *Arch. Environ. Contam. Toxicol.* 41: 208-214.

Liiri, M., Setälä, H., Haimi, J., Pennanen, T. & Fritze, H. 2002a: Relationship between soil microarthropod species diversity and plant growth does not change when the system is disturbed. – *Oikos* 96: 137-149.

Liiri, M., Setälä, H., Haimi, J., Pennanen, T. & Fritze, H. 2002b: Soil processes are not influenced by the functional complexity of soil decomposer food webs under disturbance. – *Soil Biol. Biochem.* 34: 1009-1020.

Lin, Z., Comet, B., Qvarfort, U. & Herbert, R. 1995: The chemical and mineralogical behaviour of Pb in shooting range soils from central Sweden. – *Environ. Pollut.* 89: 303-309.

Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Hogberg, P., Stenlid, J. & Finlay, R. D. 2007: Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. – *New Phytol.* 173: 611-620.

Lindberg, N. & Bengtsson, J. 2006: Recovery of forest soil fauna diversity and composition after repeated summer droughts. – *Oikos* 114: 494-506.

Lomander, A. & Johansson, M. 2001: Changes in concentrations of Cd, Zn, Mn, Cu and Pb in spruce (Picea abies) needle litter during decomposition. – *Water Air Soil Pollut.* 132: 165-184.

Lukkari, T. & Haimi, J. 2005: Avoidance of Cu- and Zn-contaminated soil by three ecologically different earthworm species. – *Ecotoxicol. Environ. Saf.* 62: 35-41.

Luo, W., Verweij, R. A. & van Gestel, C. A. M. 2014: Contribution of soil properties of shooting fields to lead biovailability and toxicity to Enchytraeus crypticus. – *Soil Biol. Biochem.* 76: 235-241.

Ma, W. 1989: Effect of soil pollution with metallic lead pellets on lead bioaccumulation and organ/body weight alterations in small mammals. – *Arch. Environ. Contam. Toxicol.* 18: 617-622.

Macfadyen, A. 1961: Improved funnel-type extractors for soil microarthropods. – *J. Anim. Ecol.* 30: 171-184.

Mälkönen, E., Derome, J., Fritze, H., Helmisaari, H., Kukkola, M., Kytö, M., Saarsalmi, A. & Salemaa, M. 1999: Compensatory fertilization of Scots pine stands polluted by heavy metals. – *Nutr. Cycling Agroecosyst.* 55: 239-268.

Manninen, S. & Tanskanen, N. 1993: Transfer of lead from shotgun pellets to humus and three plant species in a Finnish shooting range. – *Arch. Environ. Contam. Toxicol.* 24: 410-414.

Marcin, C., Marcin, G., Justyna, M., Katarzyna, K. & Maria, N. 2013: Diversity of microorganisms from forest

soils differently polluted with heavy metals. – *Appl. Soil Ecol.* 64: 7-14.
Mateo, R., Green, A., Jeske, C., Urios, V. & Gerique, C. 2001: Lead poisoning in the globally threatened marbled teal and white-headed duck in Spain. – *Environ. Toxicol. Chem* 20: 2860-2868.
Matthews, R., Landis, W. & Matthews, G. 1996: The community conditioning hypothesis and its application to environmental toxicology. – *Environ. Toxicol. Chem.*15: 597-603.
McBride, M., Sauve, S. & Hendershot, W. 1997: Solubility control of Cu, Zn, Cd and Pb in contaminated soils. – *Eur. J. Soil Sci.* 48: 337-346.
McEnroe, N. A. & Helmisaari, H. -. 2001: Decomposition of coniferous forest litter along a heavy metal pollution gradient, south-west Finland. – *Environ. Pollut.* 113: 11-18.
Menon, M., Hermle, S., Guenthardt-Goerg, M. S. & Schulin, R. 2007: Effects of heavy metal soil pollution and acid rain on growth and water use efficiency of a young model forest ecosystem. – *Plant Soil* 297: 171-183.
Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Oosterbaan, A., Tack, F. M. G. & Verheyen, K. 2007: Tree species effect on the redistribution of soil metals. – *Environ. Pollut.* 149: 173-181.
Mielke, P., Berry, K. & Johnson, E. 1976: Multi-Response Permutation Procedures for a Priori Classifications. – *Communications in Statistics Part A-Theory and Methods* 5: 1409-1424.
Migliorini, M., Pigino, G., Caruso, T., Fanciulli, P., Leonzio, C. & Bernini, F. 2005: Soil communities (Acari Oribatida; Hexapoda Collembola) in a clay pigeon shooting range. – *Pedobiologia 49(1) 2005: 1-13.* 49: 1-13.
Migliorini, M., Pigino, G., Bianchi, N., Bernini, F. & Leonzio, C. 2004: The effects of heavy metal contamination on the soil arthropod community of a shooting range. – *Environ. Pollut.* 129: 331-340.
Naumanen, P., Sorvari, J., Pyy, O., Rajala, P., Penttinen, R., Tiainen, J. & Lindroos, S. 2002: Ampumarata-alueiden pilaantunut maaperä. – *Suomen Ympäristö* 543: 282.
Newman, M. C. 2010: *Fundamentals of Ecotoxicology*. Taylor & Francis Group. Boca Raton, USA.
Niklinska, M., Laskowski, R. & Maryanski, M. 1998: Effect of heavy metals and storage time on two types of forest litter: Basal respiration rate and exchangeable metals. – *Ecotoxicol. Environ. Saf.* 41: 8-18.
Nugroho, R. A., Roling, W. F. M., Laverman, A. M. & Verhoef, H. A. 2007: Low nitrification rates in acid scots pine forest soils are due to pH-related factors. – *Microb. Ecol.* 53: 89-97.
O'Connor, F. B. 1962: The extraction of Enchytraeidae from soil. In: Murphy, P. W. (ed.), *Progress in soil zoology*: 279-285. Butterworth. London.
Owojori, O. J., Healey, J., Princz, J. & Siciliano, S. D. 2011: Can Avoidance Behavior of the Mite Oppia Nitens be used as a Rapid Toxicity Test for Soils Contaminated with Metals Or Organic Chemicals?. – *Environ. Toxicol. Chem.* 30: 2594-2601.
Park, J. H., Bolan, N. S., Chung, J. W., Naidu, R. & Megharaj, M. 2011: Environmental monitoring of the role of phosphate compounds in enhancing immobilization and reducing bioavailability of lead in contaminated soils. – *J. Environ. Monitor.* 13: 2234-2242.
Parraga-Aguado, I., Querejeta, J., Nazaret Gonzalez-Alcaraz, M. & Conesa, H. M. 2014: Metal(loid) allocation and nutrient retranslocation in Pinus halepensis trees growing on semiarid mine tailings. – *Sci. Total Environ.* 485: 406-414.
Pattee, O. H. & Pain, D. J. 2002: Lead in the environment. In: Hoffmann, D. J., Rattner, B. A., Burton Jr., G. A. and Cairns Jr., J. (eds.), *Handbook of Ecotoxicology* 2nd ed.: 373-408. Lewis Publishers. Boca Raton, USA.
Pennanen, T., Frostegård, Å., Fritze, H. & Bååth, E. 1996: Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. – *Appl. Environ. Microbiol.* 62: 420-428.
Pokras, M. A. & Kneeland, M. R. 2008: Lead Poisoning: Using Transdisciplinary Approaches to Solve an Ancient Problem. – *Ecohealth* 5: 379-385.
Post, R. & Beeby, A. 1996: Activity of the microbial decomposer community in metal-contaminated roadside soils. – *J. Appl. Ecol.* 33: 703-709.

Puglisi, E., Hamon, R., Vasileiadis, S., Coppolecchia, D. & Trevisan, M. 2012: Adaptation of Soil Microorganisms to Trace Element Contamination: A Review of Mechanisms, Methodologies, and Consequences for Risk Assessment and Remediation. – *Crit. Rev. Environ. Sci. Technol.* 42: 2435-2470.

Rantalainen, M., Torkkeli, M., Strömmer, R. & Setälä, H. 2006: Lead contamination of an old shooting range affecting the local ecosystem - A case study with a holistic approach. – *Sci. Total Environ.* 369: 99-108.

Reid, B. J. & Watson, R. 2005: Lead tolerance in Aporrectodea rosea earthworms from a clay pigeon shooting site. – *Soil Biol. Biochem.* 37: 609-612.

Robinson, B. H., Bischofberger, S., Stoll, A., Schroer, D., Furrer, G., Roulier, S., Gruenwald, A., Attinger, W. & Schulin, R. 2008: Plant uptake of trace elements on a Swiss military shooting range: Uptake pathways and land management implications. – *Environ. Pollut.* 153: 668-676.

Rohr, J. R., Kerby, J. L. & Sih, A. 2006: Community ecology as a framework for predicting contaminant effects. – *Trends Ecol. Evol.* 21: 606-613.

Rooney, C. P., McLaren, R. G. & Cresswell, R. J. 1999: Distribution and phytoavailability of lead in a soil contaminated with lead shot. – *Water Air Soil Pollut.* 116: 535-548.

Rooney, C. P., McLaren, R. G. & Condron, L. M. 2007: Control of lead solubility in soil contaminated with lead shot: Effect of soil pH. – *Environ. Pollut.* 149: 149-157.

Rusk, J., Hamon, R., Stevens, D. & McLaughlin, M. 2004: Adaptation of soil biological nitrification to heavy metals. – *Environ. Sci. Technol.* 38: 3092-3097.

Russell, D. & Alberti, G. 1998: Effects of long-term, geogenic heavy metal contamination on soil organic matter and microarthropod communities, in particular Collembola. – *Appl. Soil Ecol.* 9: 483-488.

Salminen, J., van Gestel, C. A. & Oksanen, J. 2001a: Pollution-induced community tolerance and functional redundancy in a decomposer food web in metal-stressed soil. – *Environ. Toxicol. Chem.* 20: 2287-2295.

Salminen, J., Anh, B. & van Gestel, C. 2001b: Indirect effects of zinc on soil microbes via a keystone enchytraeid species. – *Environ. Toxicol. Chem.* 20: 1167-1174.

Salminen, J., Korkama, T. & Strömmer, R. 2002: Interaction modification among decomposers impairs ecosystem processes in lead-polluted soil. – *Environ. Toxicol. Chem.* 21: 2301-2309.

Sauve, S. 2006: Copper inhibition of soil organic matter decomposition in a seventy-year field exposure. – *Environ. Toxicol. Chem.* 25: 854-857.

Sauve, S., McBride, M. & Hendershot, W. 1998: Soil solution speciation of lead(II): Effects of organic matter and pH. – *Soil Sci. Soc. Am. J.* 62: 618-621.

Sauve, S., Dumestre, A., McBride, M., Gillett, J., Berthelin, J. & Hendershot, W. 1999: Nitrification potential in field-collected soils contaminated with Pb or Cu. – *Appl. Soil Ecol.* 12: 29-39.

Scheetz, C. D. & Rimstidt, J. D. 2009: Dissolution, transport, and fate of lead on a shooting range in the Jefferson National Forest near Blacksburg, VA, USA. – *Environ. Geol.* 58: 655-665.

Scheid, S., Guenthardt-Goerg, M. S., Schulin, R. & Nowack, B. 2009: Accumulation and solubility of metals during leaf litter decomposition in non-polluted and polluted soil. – *Eur. J. Soil Sci.* 60: 613-621.

Seguin, V., Gagnon, C. & Courchesne, F. 2004: Changes in water extractable metals, pH and organic carbon concentrations at the soil-root interface of forested soils. – *Plant Soil* 260: 1-17.

Selonen, S., Liiri, M. & Setälä, H. 2014: Can the soil fauna of boreal forests recover from lead-derived stress in a shooting range area? – *Ecotoxicology* 23: 437-448.

Selonen, S., Liiri, M., Strömmer, R. & Setälä, H. 2012: The fate of lead at abandoned and active shooting ranges in a boreal pine forest. – *Environ. Toxicol. Chem.* 31 :2771-2779.

Selonen, S. & Setälä, H. 2015: Soil processes and tree growth at shooting ranges in a boreal forest reflect contamination history and lead-induced changes in soil food webs. – *Sci. Total Environ.* 518-519:320-327.

Setälä, H. 2005: Does biological complexity relate to functional attributes of soil food webs? In: de Ruiter, P., Wolters, V. and Moore, J. (eds.), *Dynamic food webs - multispecies assemblages, ecosystem*

development and environmental change: 308-320. Elsevier. Amsterdam.
Setälä, H. 1995: Growth of Birch and Pine-Seedlings in Relation to Grazing by Soil Fauna on Ectomycorrhizal Fungi. – *Ecology* 76: 1844-1851.
Setälä, H. & Huhta, V. 1991: Soil Fauna Increase Betula-Pendula Growth - Laboratory Experiments with Coniferous Forest Floor. – *Ecology* 72: 665-671.
Setälä, H. & McLean, M. 2004: Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. – *Oecologia* 139: 98-107.
Seybold, C., Herrick, J. & Brejda, J. 1999: Soil resilience: A fundamental component of soil quality. – *Soil Sci.* 164: 224-234.
Shao, Y., Zhang, W., Shen, J., Zhou, L., Xia, H., Shu, W., Ferris, H. & Fu, S. 2008: Nematodes as indicators of soil recovery in tailings of a lead/zinc mine. – *Soil Biol. Biochem.*40: 2040-2046.
Shi, W., Becker, J., Bischoff, M., Turco, R. & Konopka, A. 2002: Association of microbial community composition and activity with lead, chromium, and hydrocarbon contamination. – *Appl. Environ. Microbiol.* 68: 3859-3866.
Smit, C., van Beelen, P. & Van Gestel, C. 1997: Development of zinc bioavailability and toxicity for the springtail Folsomia candida in an experimentally contaminated field plot. – *Environ. Pollut.* 98: 73-80.
Sorvari, J. 2007: Environmental risks at Finnish shooting ranges - A case study. – *Hum. Ecol. Risk Assess.* 13: 1111-1146.
Sorvari, J., Antikainen, R. & Pyy, O. 2006: Environmental contamination at Finnish shooting ranges-the scope of the problem and management options. – *Sci. Total Environ.* 366: 21-31.
Sousa, N. R., Ramos, M. A., Marques, A. P. G. C. & Castro, P. M. L. 2014: A genotype dependent-response to cadmium contamination in soil is displayed by Pinus pinaster in symbiosis with different mycorrhizal fungi. – *Appl. Soil Ecol.*76: 7-13.
Speir, T., Kettles, H., Percival, H. & Parshotam, A. 1999: Is soil acidification the cause of biochemical responses when soils are amended with heavy metal salts?. – *Soil Biol. Biochem.* 31: 1953-1961.
Spurgeon, D. J., Lister, L., Kille, P., Pereira, M. G., Wright, J. & Svendsen, C. 2011: Toxicokinetic studies reveal variability in earthworm pollutant handling. – *Pedobiologia* 54: S217-S222.
Stansley, W. & Roscoe, D. 1996: The uptake and effects of lead in small mammals and frogs at a trap and skeet range. – *Arch. Environ. Contam. Toxicol.* 30: 220-226.
Stjernquist, I., Nihlgard, B., Filiptchouk, A. N. & Strakhov, V. V. 1998: Soil and forest vitality as affected by air pollutants on the Kola Peninsula. – *Chemosphere* 36: 1119-1124.
Stohs, S. & Bagchi, D. 1995: Oxidative Mechanisms in the Toxicity of Metal-Ions. – *Free Radical Biology and Medicine* 18: 321-336.
Swift, M. J. & Anderson, J. M. 1989: Decomposition. In: Lieth, H. & Werger, M. J. A. (eds), *Tropical Rain Forest Ecosystems. Biogoechemical and Ecological Studies*: 547-69. Amsterdam. Elsevier.
Takamatsu, T., Murata, T., Koshikawa, M. K. & Watanabe, M. 2010: Weathering and dissolution rates among Pb shot pellets of differing elemental compositions exposed to various aqueous and soil conditions. – *Arch. Environ. Contam. Toxicol.* 59: 91-99.
Tuomela, M., Steffen, K., Kerko, E., Hartikainen, H., Hofrichter, M. & Hatakka, A. 2005: Influence of Pb contamination in boreal forest soil on the growth and ligninolytic activity of litter-decomposing fungi. – *FEMS Microbiol. Ecol.* 53: 179-186.
Turner, A. & Dickinson, N. 1993: Survival of Acer-Pseudoplatanus L (Sycamore) Seedlings on Metalliferous Soils. – *New Phytol.* 123: 509-521.
Väisänen, A., Suontamo, R., Silvonen, J. & Rintala, J. 2002: Ultrasound-assisted extraction in the determination of arsenic, cadmium, copper, lead, and silver in contaminated soil samples by inductively coupled plasma atomic emission spectrometry. – *Anal. Bioanal. Chem.* 373: 93-97.
van Gestel, C. A. M. 2012: Soil ecotoxicology: state of the art and future directions. – *Zookeys*: 275-296.
Van Straalen, N. 2003: Ecotoxicology becomes stress ecology. – *Environ. Sci. Technol.* 37: 324A-330A.
Vyas, N., Spann, J., Heinz, G., Beyer, W., Jaquette, J. & Mengelkoch, J. 2000: Lead

poisoning of passerines at a trap and skeet range. – *Environ. Pollut.* 107: 159-166.
Walker, B. H. 1992: Biodiversity and Ecological Redundancy. – *Conserv. Biol.* 6: 18-23.
Wardle, D., Bardgett, R., Klironomos, J., Setälä, H., van der Putten, W. & Wall, D. 2004: Ecological linkages between aboveground and belowground biota. – *Science* 304: 1629-1633.
White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. 1979: Determination of the sedimentary microbial biomass by extractible lipid phosphate. – *Oecologia* 40: 51-62.
White, L. D., Cory-Slechta, D. A., Gilbert, M. E., Tiffany-Castiglioni, E., Zawia, N. H., Virgolini, M., Rossi-George, A., Lasley, S. M., Qian, Y. C. & Basha, M. R. 2007: New and evolving concepts in the neurotoxicology of lead. – *Toxicol. Appl. Pharmacol.* 225: 1-27.
Yeates, G. 1971: Feeding Types and Feeding Groups in Plant and Soil Nematodes. – *Pedobiologia* 11: 173-179.
Zaitsev, A. & van Straalen, N. 2001: Species diversity and metal accumulation in oribatid mites (Acari, Oribatida) of forests affected by a metallurgical plant. – *Pedobiologia* 45: 467-479.
Zhang, C., Huang, L., Luan, T., Jin, J. & Lan, C. 2006: Structure and function of microbial communities during the early stages of revegetation of barren soils in the vicinity of a Pb/Zn Smelter. – *Geoderma* 136: 555-565.